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Chemistry

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Synthesis of Selective Antagonists for the various adenosine receptors

60 study points

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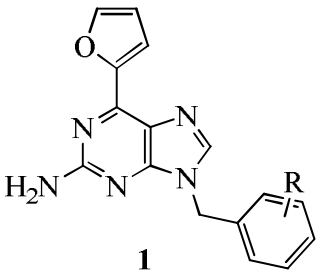
ABSTRACT

Investigation of structure- activity relationship have been explored through substitutions at the 9-position of 2-amino-6-(2-furanyl) purine, to identify novel and selective A_{2A} adenosine receptor antagonists. Several potent and selective antagonists were identified. In particular, compound **1**, with various R substituents show very high affinity with excellent selectivity[68].In this study, analogs of **2** with substituents in the purine 8-position were synthesized. targets compounds are compound **2a**, **2b**, and **2c**.



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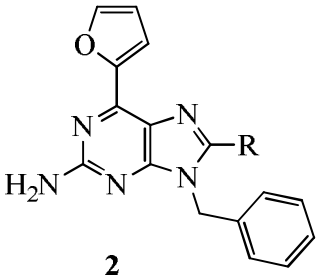

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Modification in the imidazole ring

2a: R=CH_3
2b: R=OEt
2c: R= Cl

ABBREVIATION

Ac	Acetyl
AR	Adenosine Receptor
BuLi	Buthyl lithium
cAMP	Cyclic-Adenosine monophosphate
CNS	Central nervous system
DMF	Dimethylformamide
Et al.	Et alia (and others)
EtOAc	Ethyl acetate
EtOH	Ethanol
GDP	Guanine diphosphate
GPCR	G-Protein Coupled Receptor
GTP	Guanine triphosphate
HMBC	Heteronuclear multiple bond correlation experiment
HMQC	Heteronuclear multiple-quantum coherence
MeOH	Methanol
M.P	Melting point
MS	Mass spectroscopy
NaOEt	Sodium ethaoxide
NEt_3	Triethylamine
Ph	Phenyl
R	Hydrocarbon
THF	Tetrahydrofuran
THP	Tetrahydropyran

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1. 0 Introduction

Purine (Fig. 1) is the most abundant nitrogen-containing heterocycle on earth. The bicyclic ring system can be found in many naturally occurring compounds with a wide variety of biological roles.

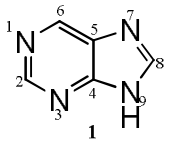

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Fig. 1. The purine ring and the accepted numbering system

Since purines are involved in so many essential biological processes, derivatives have been extensively studied as potential drugs and several purines are clinically used as drugs, especially as anticancer- and antiviral compounds.[3,4] This explains why an active research, including herein, focuses on synthesising modified purines as potential drugs or molecular tools. Development of metal-mediated reactions for C-C and C-N bond formation has facilitated the synthesis of a number of novel purines, many of them with highly interesting biological activities. Prominent among the purine nucleoside is adenosine (Fig. 2) and is identified as a major local regulator of tissue function especially when energy supply fails to meet cellular energy demand.[5]

1.1 The concept of Receptors

Receptors are site substances in the mammalian body and drug which agonise or antagonise neurotransmitters or hormones interact with it. The interaction of these drug substances is analogous to lock and key and as such those organic substances which have affinity to these receptors, activating it and leading to biological response are classified as agonist whereas those organic substances that also have affinity to these receptors but may be as a result of a bad conformation, no biological response is elicited are classified as antagonist. But on a

general note, any organic substance that have affinity to these receptors can be said to be a ligand and as such a ligand in this wise can either be an agonist or an antagonist.

1.1.1 Adenosine Receptors

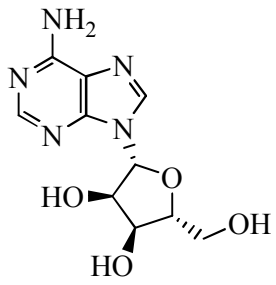

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Fig 2. Structure of adenosine

Adenosine (Fig. 2) acts as a neurotransmitter in the mammalian nervous system. Because of its polar nature and its extremely short half life, most time it is administered intravenously for the treatment of some certain acute cardiac condition. The potential of adenosine receptors as drug targets was first reviewed in 1982.[6] Significant progress has occurred over the last few years.[7] The regulatory role for adenosine implicates adenosine based drugs as therapeutic targets.[6] An improved understanding of the physiology, pharmacology and molecular biology of adenosine and adenosine receptors has taken place in recent years. This understanding is fundamental to the realisation of the therapeutic potential for this nucleoside and provides a solid foundation for the continuation of active research in the adenosine field. Adenosine is a signalling substance, which mediate its effects by activation of four different G-protein-coupled receptors (GPCR) (A_1, A_{2A}, A_{2B} and A_3).[8,9] The adenosine receptors (ARs) belong to the rhodopsin family of the GPCR super-family. GPCR is a large membrane bound protein which mediates its effects by the activation of an internal guanine nucleotide.[10] The extracellular region of the protein is composed of the amino terminus and several loops, which comprise the ligand-binding site. The carboxyl end of it is located in the part of the protein that protrudes inside the cytoplasm.[10] The ARs differs in their affinity for adenosine as well as their downstream signalling pathways activated in the target cells.

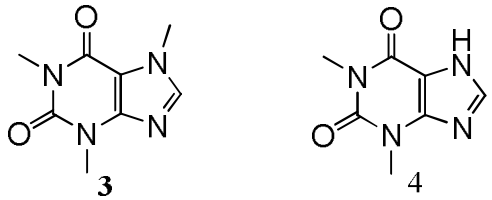
The characteristics of the α subunit are what determine the designation of the G protein. Receptor activation leads to a conformational change in the associated G protein, triggering the release of bound GDP from the α subunit, which is then replaced by a molecule of GTP.[10] With the binding of GTP, the α-subunits GTP complex dissociates from the αβ subunits and

binds to a particular target enzyme, resulting in its activation or inhibition.[10] Within a short period of time, the α subunit catalyzes the dephosphorylation of the associated GTP molecule to GDP, resulting in the reassociation of the α subunit with the αβ subunits and, thus, the return of the G protein to the inactivated state.[10]

1.1.2 Characterisation of adenosine receptor subtypes

Four adenosine receptor subtypes have now been characterised pharmacologically, structurally and functionally. These are; A_1, A_{2A}, A_{2B}, and A_3.[10] They are widely distributed in various tissues of the body. The A_1, A_{2A} and A_{2B} subtypes were initially discovered and classified in the classical manner (i.e., by a study of agonist pharmacology).[10] Evidence from recent cloning, sequencing and expression of each of these subtypes has provided structural and functional confirmation of their original classification as distinct adenosine receptor subtypes. In contrast, the A_3 receptor subtype was discovered by molecular biology studies, this was then followed by classical pharmacological studies. All four adenosine receptor subtypes have recently been cloned from a variety of mammals, including human.[11] Molecular biology has made significant contributions to adenosine receptor characterisation allowing determination of actual receptor protein primary sequences, which is fundamental to studying receptor structure, function and regulation at the molecular level.[12]

1.1.3 Physiological significance of endogenous adenosine



3 caffeine 4 theophylline

Figure 3: Naturally occurring xanthines

The initial realisation of the physiological significance of adenosine was in 1929.[13] Pronounced physiological effects were observed on both cardiovascular and renal function

following administration of adenosine to mammals. The clinical evaluation of adenosine in man proved disappointing due to the short half-life of adenosine.[13]

The ability to study further the physiological role of endogenous adenosine in a variety of mammalian tissues followed the discovery that xanthines; caffeine and theophylline, were adenosine antagonists.[14] Adenosine is now known to regulate a diverse range of physiological functions, to the extent that almost all mammalian organ systems are affected by adenosine.[15] The physiological responses to adenosine are complex and depend on the receptor subtype activated, the mammalian species, and the type and metabolic state of the tissue.[16]

A consequence of the global homeostatic function of endogenous adenosine is the multitude of physiological responses mediated by adenosine, presented in Table 1. These responses generally lead to a decrease in the oxygen demand and/or increase in the oxygen supply, reinstating the energy supply: demand balance within the tissue. One of the most important functions of endogenous adenosine is as a cytoprotective agent, preventing tissue damage during traumas such as hypoxia, ischaemia, hypotension and seizure activity.[26-30]

Table 1. The physiological effects mediated by endogenous adenosine in various mammalian tissues, attributed to activation of A_1 and A_{2A} receptor subtypes.

System	**Physiological effect of endogenous adenosine**
CNS	Neuromodulator mediating central inhibitory effects, inhibits release of neurotransmitters, inhibits neuronal firing, CNS depressant: decreases locomotor activity, anticonvulsant, sedation, hypnotic, antinociceptive, ataxic, mediates respiration.[1,2]
Kidney	Biphasic modulation of renin release,[12] decreased glomerular filtration rate by vasoconstriction
Adipocytes	Inhibits lipolysis (maintenance of body weight).[48,49]
Immune	Immunosuppressant,[50,51] inhibits lymphocyte

	proliferation, anti-inflammatory: Modulating neutrophil function.
Liver	Regulates hepaic blood flow,[52] stimulates gluconeogenesis,[59,60]
Stomach	Inhibits gastric secretion.[36,37]
Straited/smooth muscle	Relaxation.[38,39]

The A_{2B} receptor subtype is a low affinity receptor, adenosine exhibiting activity at this subtype at concentrations greater than 10 μM.[17,18] There is little information on the physiological significance of the A_{2B} subtype, a consequence of the lack of suitably potent and selective ligands for detailed study.[19] A speculative role for the A_{2B} receptor has been proposed.[20] It was suggested that the A_{2B} receptor functions during life threatening systems failure, reactivating the heart and brain, in order to resuscitate. This action would override the protective functions afforded by the A_1 and A_{2A} receptors. More recent studies have demonstrated that activation of A_{2B} receptors leads to increases in intracellular calcium concentrations in cultured cells and chloride ion secretion via cAMP in intestinal epithelial cells.[21,22] The latter has implications for the treatment of secretory diarrhoea associated with inflammation.[22]]

The physiological role for the A_3 receptor is not adequately understood, a consequence of its relatively recent characterisation and a lack of truly selective ligands for *in vivo* studies. Suitably selective and potent ligands (both agonists and antagonists) are being developed predominantly by radioligand binding studies, however the relationship between the radioligand binding data and selectivity *in vivo* is not yet established.[20 - 23] As well, ligands which are already well characterised pharmacologically at the A_1 and A_{2A} receptors are being characterised at the A_3 receptor.[24,25] So far this subtype is implicated in mediating a number of physiological responses.

The conclusion from these studies was that the A_3 receptor may play a role in mediating asthmatic attacks and other allergic responses, however other adenosine receptor subtypes have not been ruled out in contributing to the observed responses.[26] Using the A_3 selective, high affinity agonist N^6-(3-iodobenzyl)-5′-*N*-methylcarboxamidoadenosine (3-IB-MECA), *in vivo* studies in mice demonstrated the A_3 receptor mediates a locomotor behavioural

depressant effect, possibly centrally mediated.[31] A cardioprotective role for A_3 receptors, activating ischaemic preconditioning, has been proposed based on studies in isolated rabbit heart.[32] N^6-2-(4-aminophenyl)ethyladenosine (APNEA) produced a hypotensive response in the pithed rat, which was attributed to activation of A_3 receptors.[33] It was subsequently demonstrated that this A_3 receptor activation resulted in mast cell degranulation and histamine release, implicating the mast cell with a key role in A_3 receptor mediated hypotension in the rat.[34, 35]

1.1.4 Therapeutic potential for drugs acting at adenosine receptors

The diverse physiological functions of adenosine, outlined earlier, highlight the significant benefits of developing therapeutics for the regulation of adenosine receptors. The vast amount of effort invested in adenosine research is driven by the therapeutic potential for drugs which elicit their actions at adenosine receptors. However, the ubiquitous distribution of adenosine receptors in mammalian cell types, the existence of at least four distinct subtypes together with the variability of physiological responses means that exploiting this potential requires agonists and antagonists that are highly selective in their action (with respect to receptor subtype and tissue type) to be of value as therapeutics.

Successful development of therapeutics is far from trivial. Many promising ligands have been identified in the pursuit of therapeutic agents; however side effects due to low selectivity have precluded clinical development. The recent cloning of each of the adenosine receptor subtypes has allowed, and will continue to allow, the study of receptor subtype structure at the molecular level. These studies may identify the discrete differences between subtypes in terms of ligand binding requirements, and may prove fundamental to the successful design of not only potent ligands but also subtype selective ligands.

The physiological function of adenosine in the central nervous system (CNS) has been extensively researched during the last two decades. Adenosine has been described as a neuromodulator, possessing global importance in the modulation of the molecular mechanisms underlying many aspects of brain function by mediating central inhibitory effects.[16,41] An increase in neurotransmitter release follows traumas such as hypoxia, ischaemia and seizure activity. These neurotransmitters are ultimately responsible for neural degeneration and neural death, which causes brain damage or death of the individual. The development of adenosine A_1 agonists which mimic the central inhibitory effects of adenosine

(and so inhibit neurotransmitter release) may therefore be clinically useful as neuroprotective agents.[42,43] Adenosine has been proposed to be an endogenous anticonvulsant agent, inhibiting glutamate release from excitory neurons and inhibiting neuronal firing.[44] Adenosine agonists therefore have potential clinical applications as antiepileptic agents. Alkylxanthines, which are adenosine antagonists, stimulate the activity of the CNS and have proven to be effective as cognition enhancers.[45, 32] This is the joint action of antagonism of the sedative effects caused by adenosine and of increasing cerebral blood flow, thus increasing glucose and oxygen availability to the brain.[16] Selective antagonists may have therapeutic potential in the treatment of various forms of dementia, for example in Alzheimer's disease. The pathological hallmark of Parkinson's disease is the depletion of dopamine in the striatum, as a consequence of the degeneration of the substantia nigra.[40,42] With the knowledge that adenosine inhibits the release of dopamine from central synaptic terminals and that A_2 agonists reduce locomotor activity it was hypothesised that A_2 antagonists might increase the release of dopamine and consequently improve Parkinsonian symptoms. Theophylline **(4)**, in low doses, produced significant improvements in symptoms of patients with Parkinson's disease, and was proposed as a safe adjunct in the therapy of Parkinson's disease.[46] The central activities of adenosine are also implicated in the molecular mechanisms underlying sedation, hypnosis, schizophrenia, anxiety, pain, respiration, depression and substance abuse. Drugs acting at adenosine receptors therefore have therapeutic potential as sedatives, muscle relaxants, antipsychotics, anxiolytics, analgesics, respiratory stimulants and antidepressants.[43]

Adenosine modulates many aspects of renal function, including renin release, glomerular filtration rate and renal blood flow. It plays a role in mediating the haemodynamic changes associated with acute renal failure.[54] Xanthines, which antagonise the renal affects of adenosine, have potential as renal protective agents. Theophylline **(4)** has been shown to improve renal function in humans, preventing acute renal failure caused by iodine-containing radiographic contrast media used in X-ray examination. It has also been shown to improve renal function after kidney transplantation.[55] The xanthine antagonist 1,3-dipropyl-8-(3-noradamantyl)xanthine (KW-3902), is currently undergoing clinical trials as a renal protective agent.[56]

1.2 Structure – activity relationships for ligands at adenosine receptors

The primary mechanism for studying adenosine receptors, prior to the recent use of molecular biology techniques, has been by pharmacological means. A large number of ligands have been synthesised and evaluated for adenosine receptor binding affinity. Due to the large number of compounds, particularly for the A_1 and A_{2A} subtypes, a quite detailed compilation of structure–activity relationships has been acquired. These extensive structure–activity relationships have enhanced the understanding of the binding domains of adenosine receptors, highlighting the key structural features required for receptor affinity and subtype selectivity. Also, these structure–activity relationships, together with molecular modelling techniques, have been used in the development of a pharmacophore of the ligand binding characteristics.

With the discovery of the A_3 receptor, structure–activity relationships have become more complex to interpret and many existing ligands have yet to be evaluated at this subtype. A_3 evaluation is necessary in order to have a complete receptor binding profile for a particular ligand. The A_{2B} receptor is not well characterised in terms of structure–activity relationships, with just two major structure–activity studies appearing in the literature.[57,58] Although these studies were known to be at an A_2 receptor, it was not until several years later that the receptor was characterised as the A_{2B} subtype.[18] The recent cloning of the A_{2B} receptor should facilitate its future pharmacological study.

1.2.1 Adenosine receptor agonists

There exists no novel adenosine receptor agonist structure. All agonists are closely related in structure to the endogenous ligand, adenosine **6** (Fig. 2). Structural and stereochemical requirements for the ribose moiety of adenosine agonists are strict. Alterations of either structure or stereochemistry result in a loss of receptor binding potency and possibly intrinsic activity.

1.2.2 Adenosine Receptor Antagonist

In contrast to agonists, adenosine receptor antagonists are novel in structure compared to adenosine. A comparison of different classes of antagonists demonstrates that, although diverse in structure, they do share some common structural features. In general the structures are: planar, aromatic or π electron rich; and nitrogen-containing heterocycles.[7] There are

exceptions to these generalisations, including benzo[*b*]furan[61] and tetrahydrobenzothiophenone[62] derivatives, with oxygen and sulfur containing heterocycles, respectively. The heterocycles are most often 6:5 fused bicyclics or 6:6:5 fused tricyclics, substituted with hydrophobic substituents. Additionally, antagonists lack the ribose moiety which is essential for agonist activity.

The first adenosine receptor antagonists reported were the naturally occurring xanthines; caffeine **(3)** and theophylline. **(4)**[14] They exhibited weak affinity and subtype selectivity at adenosine receptors. A multitude of xanthines have since been synthesised in the quest for potent and selective ligands, and this class have now been optimised to the extent that both potent and selective A_1 and A_{2A} antagonists exist. More recently xanthines have been evaluated at the A_3 receptor.[23,24] A_3 affinity is dependent on species,[63] hence structure–activity relationships become more complex. A_3 species differences will be discussed separately.

As is the case for agonists, potent and selective xanthine antagonists stem from multiple substitutions of the parent heterocycle. Substitutions at N-1, N-3, N-7 and C-8 of xanthine contribute most to potency and selectivity at A_1 and A_{2A} receptors.[64-66] The structure–activity relationships for the N-1 and N-3 substituents of xanthines have been extensively studied at A_1 and A_{2A} receptors[64-66] and to a lesser extent at A_3 receptors.[23-26] Alkylation at both N-1 and N-3 of xanthines is required to maximise adenosine receptor binding affinity at both the A_1 and A_{2A} receptor subtypes, the rank order of potency at both subtypes is methyl < ethyl < *n*-propyl ≤ *iso*butyl. At the rat A_3 receptor 1,3-disubstituted xanthines exhibit poor affinity.[23, 24]

The most significant enhancements in affinity and subtype selectivity come with substitution of the C-8 position of xanthines. C-8 substitution combined with N-1 and N-3 (and sometimes N-7) substitution has led to the development of potent and selective A_1 and A_{2A} xanthines. Potent and selective A_{2A} xanthine antagonists were lacking until recent years. 8-Styryl derivatives of 1,3-dimethyl xanthines were found to be highly A_{2A} selective.[67]

structurally diverse non-xanthine adenosine antagonists have been identified.[7] Unlike xanthines the structure–activity relationships for these novel classes of antagonists are not well defined, nor have they been optimised to achieve maximal adenosine receptor binding affinity or subtype selectivity. Those that are selective are generally A_1 selective, with few A_{2A} selective ligands.

1.3 Approaches, hypotheses and choice of method:

This section deals with previous study, the present study and some synthetic strategies explored in the proposed project.

1.3.1 Initial research

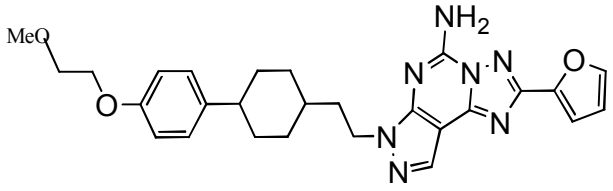


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Fig. 4. $A2_A$ antagonist (SCH-4208147)

The A_{2A} AR antagonist SCH-420814 **5** (Fig. 4) is a water-soluble analogue of SCH 58261 **6** (Fig. 5). In compound **7** (Fig. 5), the tricyclic core in SCH 58261 **6** (Fig. 5) is replaced by a purine ring, still carrying an amino-, a 2-furyl and an arylalkyl group. Several compounds **7** compared favorably to compound **6** as A_{2A} AR antagonists with respect to potency and selectivity (only binding to A_1 and A_{2A} were reported).[68] Compounds **7** are chemically closely related to 6-aryl-9-benzylpurines examined by our group as selective inhibitors of *Mycobacterium tuberculosis* (*Mtb*) *in vitro*.[69] Some of the most active antimycobacterials are shown in **8** (Fig. 5). It is worth noting that the 6-aryl-9-benzylpurines developed in the antimycobacterial project in general displayed very low toxicity towards mammalian cells.[69]. The present study now focused on synthesizing improved selective ligands which are structurally related to compound **9.** Analogues of compound **9** with Cl, Me and EtO at the 8-position are the target compounds.

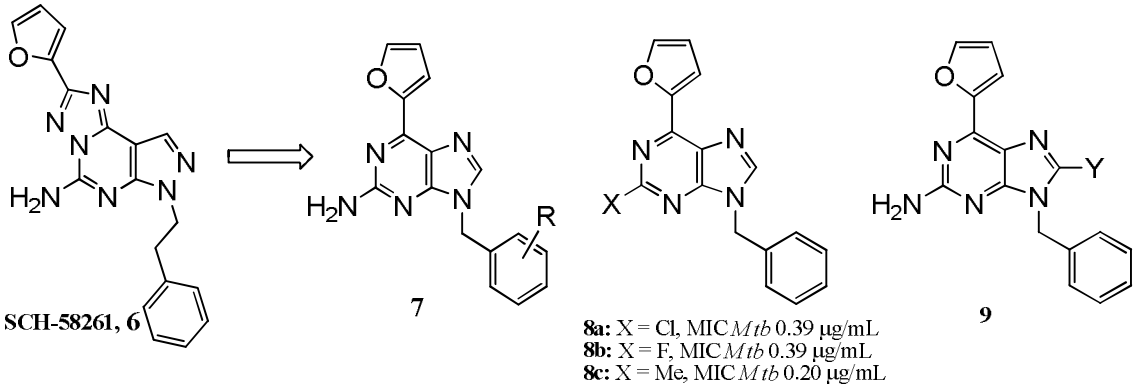


Fig. 5. Structural relationships between A_{2A} antagonists **6** and **7**, antimycobacterial compounds **8** and target compounds **9.**

1.3.2 Synthetic strategies

Synthesis was based on literature methods. The 6-aryl substituent was conveniently introduced by Pd-catalyzed coupling reactions.[69f,71] In the present study, C-8 functionalisation of purine intermediates was focused on lithiation and subsequent reaction of lithiated species with an electrophiles. Introduction of halogen this way allows further functionalization.[69i,69f,72] However, for instance purine nucleosides are readily lithiated at C-8,[72] the reaction may be more sluggish with other N-9 substituents.[71e] N-9 benzylation was possible by N-alkylation using benzylchloride.[89]

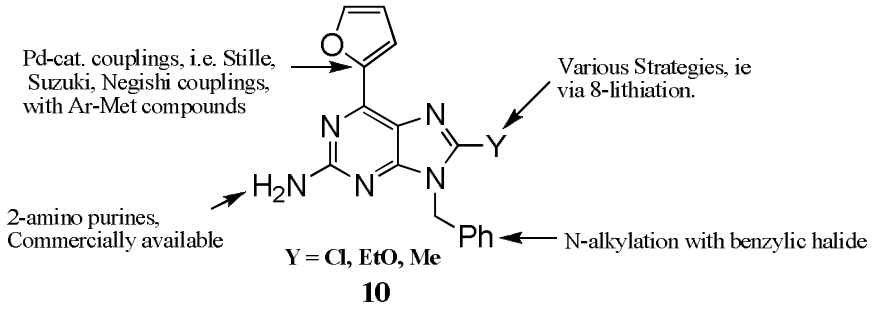
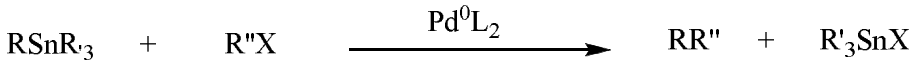


Fig. 8. Reaction commonly used in synthesis of targets compounds

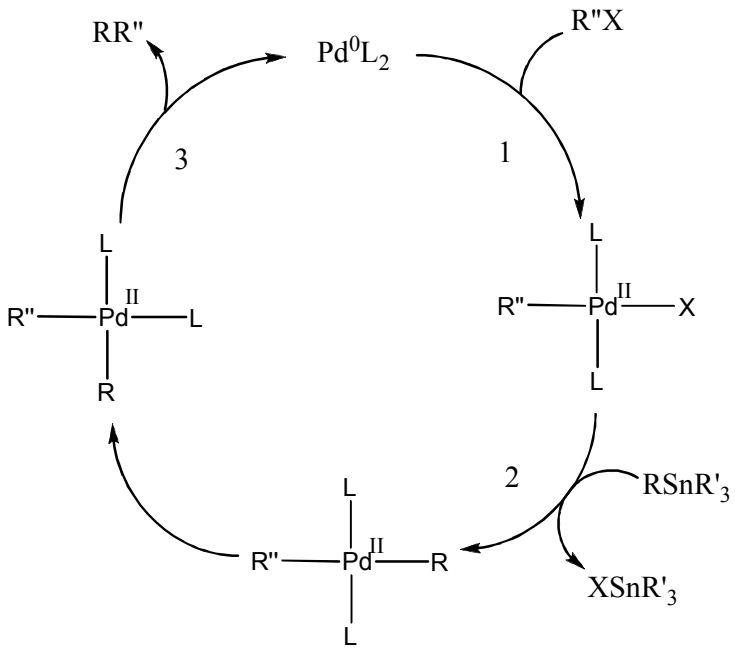
1.3.3 The Stille reaction

This reaction, discovered by John K. Stille in the 1970's eventually became known by his name, for the coupling of organostannanes with organic electrophiles. The Stille reaction has established itself as one of the two most general and most selective palladium-catalysed cross-coupling reactions along with the Suzuki cross-coupling of organoboron compounds.[73] For the synthesis of complex molecules, the Stille coupling is usually superior, displaying high selectivity and broad scope. Its tolerance towards most functional groups makes the Stille coupling particularly effective for transformations of highly functionalized molecules.[74] The Stille reaction is related to other cross-coupling reactions. Besides the two prominent members already mentioned, the family of palladium-catalyzed cross-coupling reactions includes the Hiyama,[75-78] Sonogashira,[79] Kumada,[80] and Negishi reactions, [81]



Scheme 1. The Stille reaction.

In his influential review of 1986,[82] Stille proposed a mechanism for the Stille reaction based primarily on data obtained from the coupling of benzoyl chloride with tributyl(phenyl)stannane.[82]



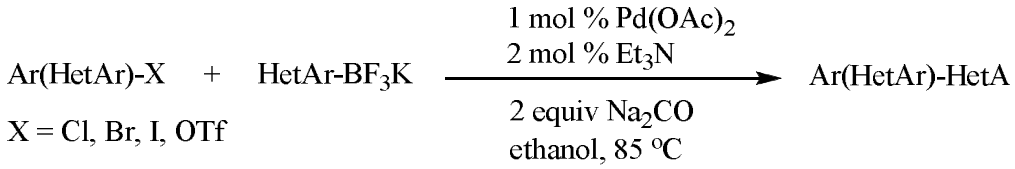
Scheme 2. Generally accepted mechanism of the Stille reaction.[83-86]

1. In the generalized mechanism, the active catalytic species was assumed to be palladium^0 (PdL_2) (L = PPh_3) complex, which undergoes oxidative addition with the organic halide R-X to form organopalladium^{II} (Scheme 2).
2. The organopalladium^{II} undergoes transmetalation with the organostannane (tetraorganotin) compound to give a diorganopalladium^{II} compound and triorganotin halide.
3. A trans to cis isomerisation, thought to be very fast, was then required for the reductive elimination to give the organic product R-R. In this process, palladium^{II} is reduced to palladium^0

1.3.4 The Suzuki – Miyaura Cross-Coupling Reaction.

The Suzuki–Miyaura cross-coupling reaction, which involves the coupling of an organoboron compound with an electrophile possesses notable advantages over the other related techniques. Particularly striking is wide range of functionality tolerated in the coupling process. The use of organoboron compounds is also valued because the inorganic by-products of the reaction are nontoxic and can be readily removed by simple workup procedures, while many tin compounds are toxic, and complete removal of tin-containing by-products are a well-recognized problem.

Because of their commercial availability, boronic acids are most often utilized in Suzuki–Miyaura cross-coupling reactions. However boronic acids exhibit several limitations that make them unattractive nucleophilic coupling partners. Boronic acids are not monomeric materials, but rather exist in equilibrium with dimmers and cyclic trimers (boroxines).[101] consequently, many boronic acids are waxy solids that are difficult to purify. Most importantly, many boronic acids, and especially electron-deficient heteroarylboronic acids, have a short shelf life due to their tendency to undergo facile protodebronation. This instability often requires their storage at low temperatures. The tendency to protodeboronate quite often manifests itself during cross-coupling reactions carried out in polar protic solvents.[102] The protodeboronation influences the stoichiometry of the reaction.

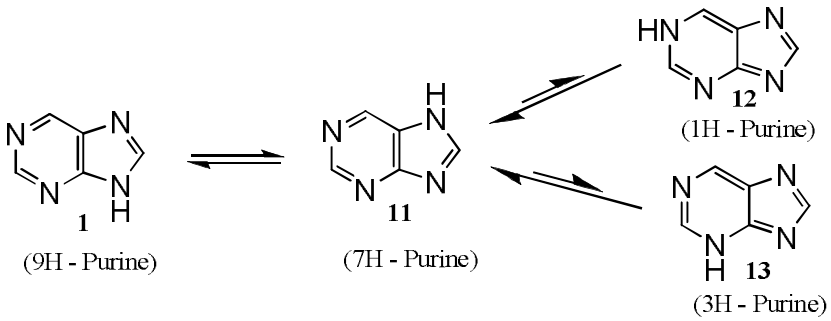


Scheme 3

In this study, a greener approach which employs the use of potassium organotrifluoroborates as coupling partners for the Suzuki – Miyaura reaction[103] (Scheme 3) was attempted.

1.3.5 N-Alkylation of purines.

The unsubstituted purine can exist in four possible tautomers (Scheme 4). The ratio of these tautomers depends on the state of the purine. In crystalline state, it exists as a 7H-tautomer. Both 7H- and 9H-tautomers exist in approximately equal amounts in solution; while 1H- and 3H tautomers are insignificant.[90]



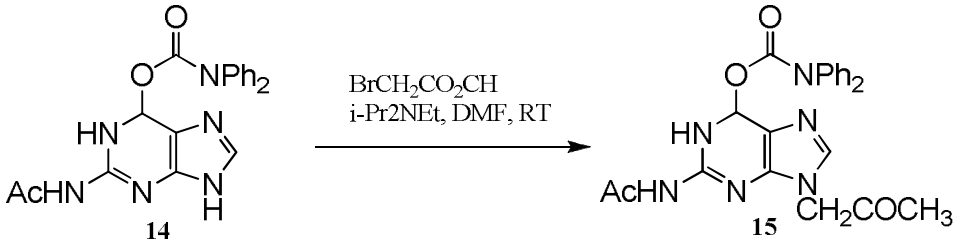
Scheme 4. Different tautomers of purines

Using a base enhances the reaction of purine nitrogen with electrophilic alkylating agents. The purine N-hydrogen is acidic and can be removed by a base forming a more reactive species. The anionic form can exist in any of the four tautomers (Scheme 4).

Alkylation can take place on any of the ring atoms. The position of alkylation is however affected by the substituent on the ring atom and the nature of the alkylating agent. For instance adenine gives 3-alkylated products under neutral conditions but 7/9-substitution results under basic conditions[89] while adenosine derivatives usually give the 1-alkylated products which is assumed to be due to hindrance to the N-3 position.[90] From this example, it can be deduced that with electron donating group in the six member ring, the ring is activated

towards electrophiles. But with electron withdrawing group in the six member ring for example the 6-chloropurines, reactivity is directed to the five member ring forming the N-7 and N-9 products the ratio of which depends on the alkylating agents. The major product in most cases is the N-9substituted product. Benzyl halide alkylating agents give mainly the N-9 product as the major product for 6-chloropurine[90] which is assumed to be due to steric hindrance from the 6-chloro group which interferes the formation of N-7 alkylated isomer. This goes to say that the ratio of N-9 to the N-7 alkylation is greatly influenced by the size of the substituent in the 6-position.[90]

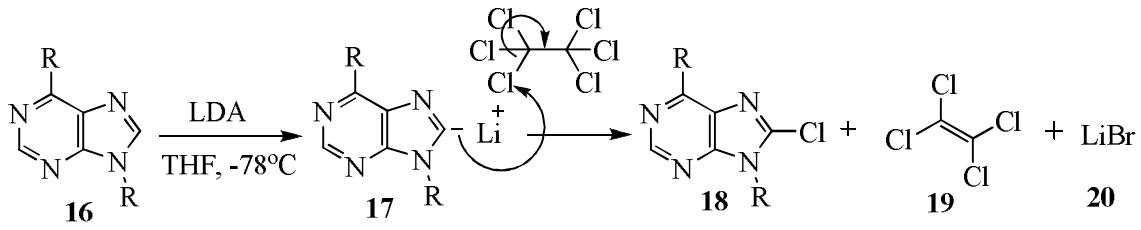
In difficult situation where N-7/N-9 selectivity is poor, alkylation can be directed to the N-9 by using a bulky protecting group in the C-6 position Scheme 5.[90]



Scheme 5.

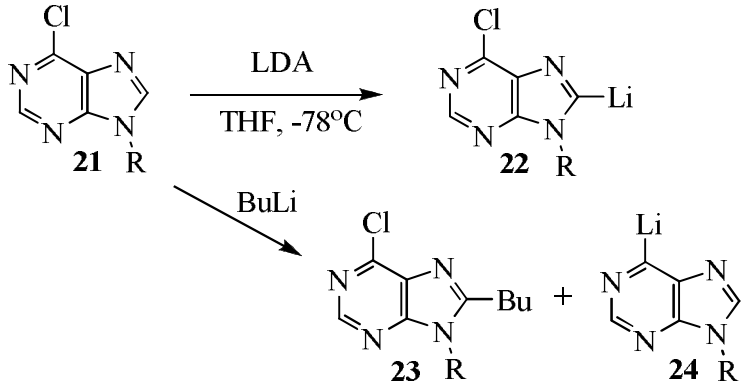
1.3.6 Lithiation-based electrophilic substitution at the purine 8-position

Purine 8- position is unreactive towards electrophiles. However, purinyl anions derived via lithiation can react readily with electrophiles like hexachloroethane (Scheme 6). 8-halo purines accessible by this route can be used for further functionalization like nucleophilic substitution.[91-94] Alkylation at 8-position can also be achieved by reaction of organolithium intermediates with alkylating agents e.g. iodomethane. The lithiation is mostly carried out using lithium amide.



Scheme 6

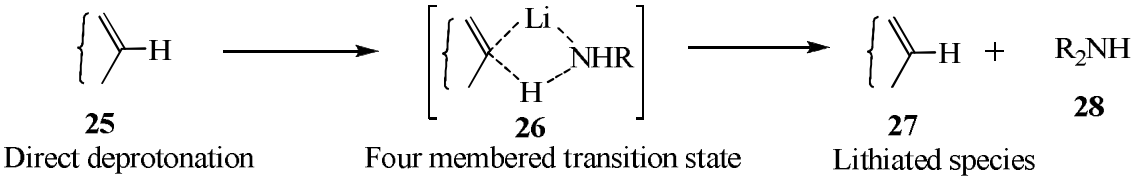
Lithium amide compounds particularly lithium amides $(R_2NLi)n$, are commonly used as strong bases in organic synthesis. The ability of these species as reagents, especially where the organic groups (R) are bulky, relies mainly on their low nucleophilicity compared with C-Li bonded complexes (e.g. MeLi). These properties cause proton abstraction to be favoured over nucleophilic addition. Such deprotonation reaction can also be regio and/or enantiospecific.[95] One of such commonly used lithiation agents is lithium diisopropylamide (LDA). LDA has been indicated by Kato et al.[96] and Tanaka et al.[97] as a better lithiating agent compared with butyllithium for regioselective lithiation at position-8 (illustrated in Scheme 7). It can cause lithiation in position-8 even in the presence of a halogen (e.g. chlorine) in the 6-position. When butyllithium was used for the same reaction there was nucleophilic addition of butyl to form 8-butylated purine and a lithium-halogen exchange forming a butyl chloride and a 6-lithiated purine.[98]



Scheme 7

The exact mechanism of the metallation (lithiation) is not known, but it is thought to involve a four centre transition state (Scheme 8).[90] In the lithiation process although a 'free anion' is never formed, the ease of lithiation correlates well with the C-hydrogen

acidity and of course this, with the stability of the corresponding conjugate base (carbanion).[90]



Scheme 8

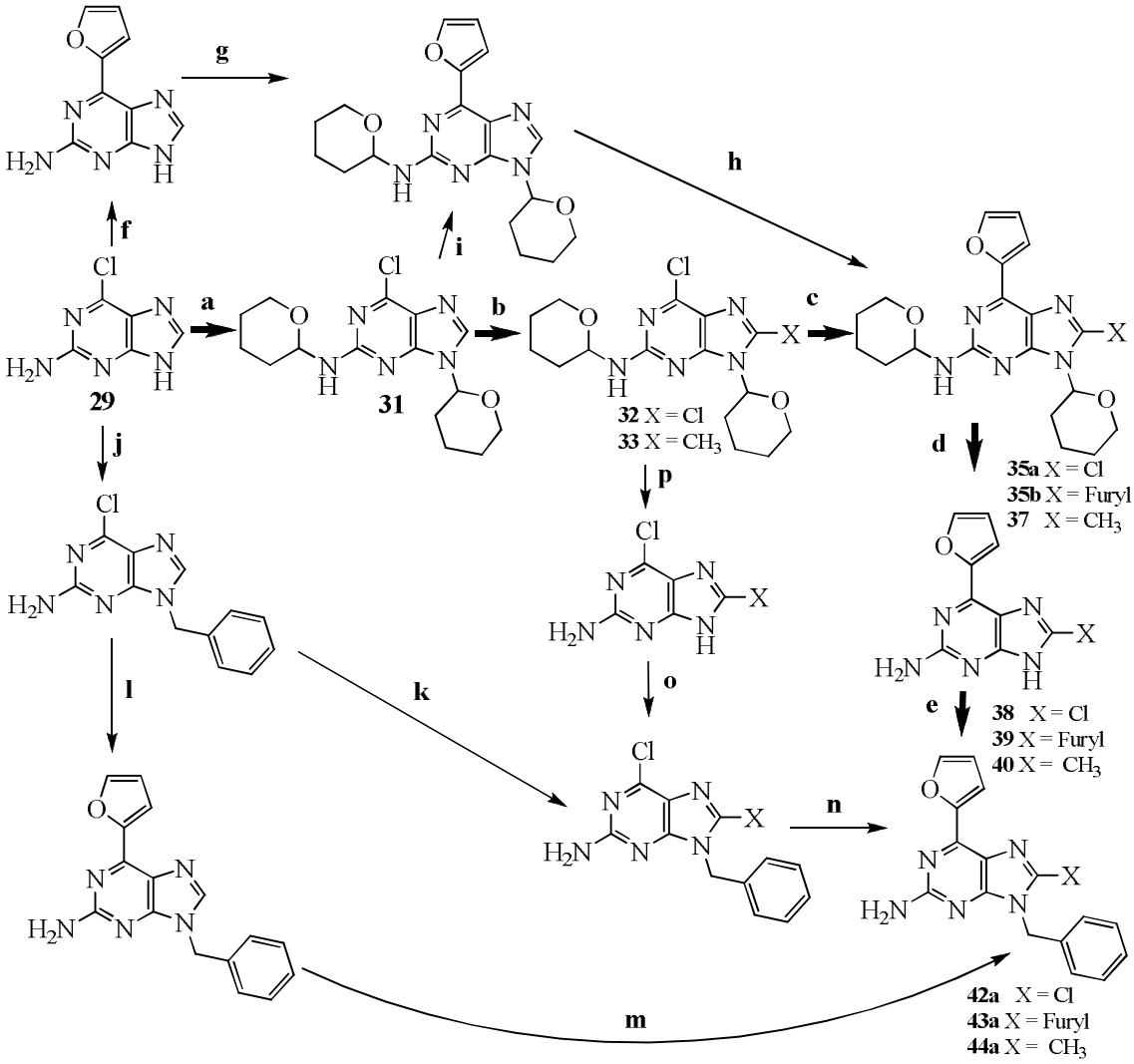
The acidity of N-9 and N-7 hydrogen makes it important to protect this position before lithiation (using strong base such as LDA) can take place at C-8.[90] This however does not seem to be always the case since all attempts made by Nolsøe *et al*. to lithiate 6-chloro-9-benzypurine failed.[100] Therefore attached substituents in the imidazole ring depending on their nature can affect the C-hydrogen acidity in the ring either by inductive or mesomeric effect.[99]

2.0 RESULTS AND DISCUSSION

2.1 SYNTHESIS OF TARGET MOLECULES.

Synthesis of the following target molecules is discussed in this section

- 9-Benzyl-6-(furan-2-yl)-8-methyl-9*H*-purin-2-amine **(44a)**
- 9-Benzyl-8-chloro-6-(furan-2-yl)-9*H*-purin-2-amine **(42a)**
- 9-Benzyl-6,8-di(furan-2-yl)-9*H*-purin-2-amine **(43a)**
- 9-Benzyl-8-ethoxy-6-(furan-2-yl)-9*H*-purin-2-amine **(45)**



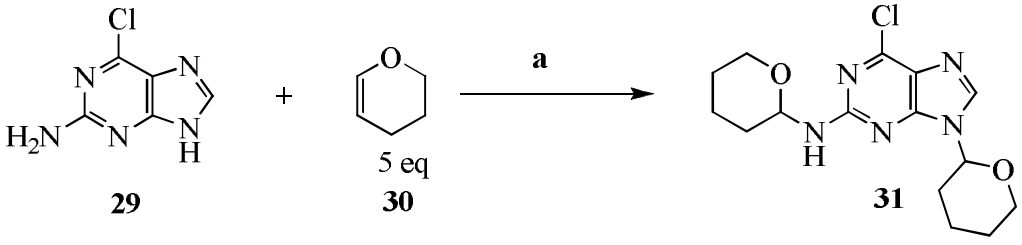
Scheme 9. Possible Routes to synthesis target compounds.

Above (Scheme 9) shows roughly, the possible routes to approach this synthesis starting from the commercially available 6-chloro-9*H*-purin-2-amine (**29**). Among these possible synthetic routes to the target compounds, routes with thick black arrows were preferred because of the following reasons

- Routes **f** and **i** were avoided because Synthesising compounds **35a** and **37** via route **h** may lead to lithiation in the furyl ring which was observed as earlier reported.[69i]
- Route **j** was avoided because routes **k** and **m** may be unsuccessful since all attempts made by Nolsøe *et al.* to lithiate 6-chloro-9-benzylpurine failed.[100]
- Route **e** was preferred to route **o** because the 6-fuyl substituents which is larger in size than the 6-chloro substituents may influence the increase in the percentage of 9- *versus* 7-alkylation.[90] This was the reason why route **p** was not attempted.

2.1.1 6-Chloro-*N*,9-bis(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-2-amine (31).

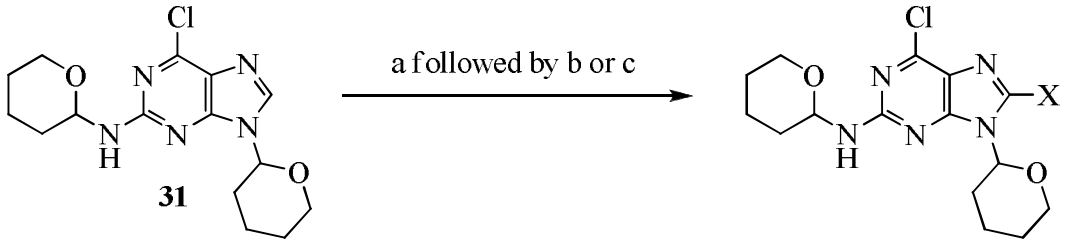
As stated ealier, the acidity of *N*-9 and the amino hydrogens makes it important to protect this positions before proceding with lithiation. This was achieved by reacting 6-chloro-9*H*-purine-2-amine **(29)** with 3,4-dihydro-2*H*-pyran **(30)** using HCl in DMF as catalyst (Scheme 10). The product compound (**31)** was isolated in moderate yield 55% compared to the yield reported in literature 71%.[104] The reaction conditions, solvent, temperature, time, etc, chosen for this reaction were the same as those used in earlier report.[104]



Scheme 10. Reagents and conditions: a – 2M HCl in DMF (0.1 eq,), DMF, 60^{o}C, 2 hr

2.1.2 6-Chloro-8-methyl-*N*,9-bis(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-2-amine (32) and 6,8-dichloro-*N*,9-bis(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-2-amine (33).

6-Chloro-*N*,9-bis(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-2-amine (**31**) was lithiated at C-8 using LDA and trapped with methyl iodide or hexachloroethane[69i] to give compounds **32** and **33** respectively. These reactions yielded only the desired products (Scheme 11).



32 X = CH_3, 90%
33 X = Cl, 91%

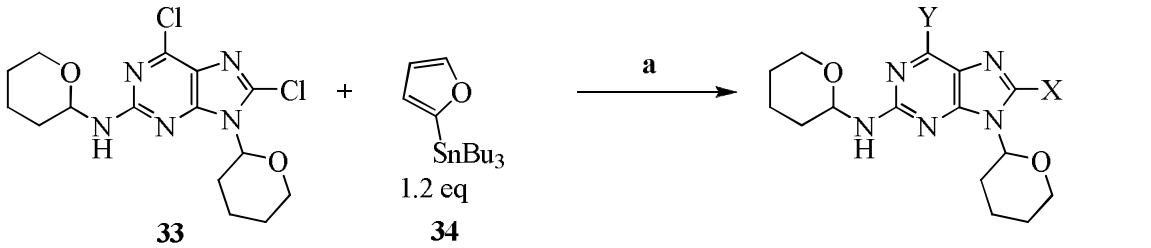
Scheme 11. Reagents and conditions: (a) LDA, THF, -78 ^oC; (b) CH_3I (10 eq), THF, -78 ^oC (c) C_2Cl_6 (2 eq), THF, -78 ^oC.

2.1.3 8-Chloro-6-(furan-2-yl)-*N*,9-bis(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-2-amine (35a).

Compound **33** was subjected to Stille coupling using Pd_2dba_3 and 2-furyl)3P as catalyst to give the furylpurines (**35**) (Scheme 12). Complete conversion was not obtained under mild conditions,[69f] also moderate amount of dicoupled product **35b** was formed. The ^1H NMR spectrum of the crude reaction mixture showed the distribution to be **35a** : **35b** : **33**; 4 : 2 : 1 and the products **35a** : **35b** : **33**; were isolated in 43%, 19% and 13% yield respectively. Increase in temperature resulted in better conversion at expense of selectivity (Table 2) as compounds **35a** and **35b** were formed in almost in the ratio of 1:1 as judged from the crude NMR. We envisioned that sterical hindrance near C-8 position would push the selectivity towards C-6 if a bulkier ligand like $PdCl_2$(dppf) is used. But the increase in yield was not significant (Table 3).

The tributyl tin chloride (Bu_3SnCl) present in the crude reaction mixture can be removed by stirring the crude mixture with an alcoholic solution of KF, leading to the formation of

insolouble trialkyl fluorides,[88,87] which can then be removed by flash chromatography. KF-MeOH treatment was avoided during work-up of this synthesis because the product **35a** contains a good leaving group which can result to nucleophilic substitution and the introduction of a methoxy group.[89,70] KF-THF treatment was rather preferred. Despite this attempt, tributyl tin chloride formed in this transformation could not be completely removed. In further attempts, crude reaction mixture was partitioned between MeCN and hexane, repeated washings of MeCN layer with hexane allowed removal of lipophilic tributyl tin chloride.



33 **34**

35a X = Cl, Y = Furyl,
35b X = Furyl, Y = Furyl
33 X = Cl, Y = Cl, Recovered

Scheme 12. Reagents and conditions. a- Pd Cat. DMF.

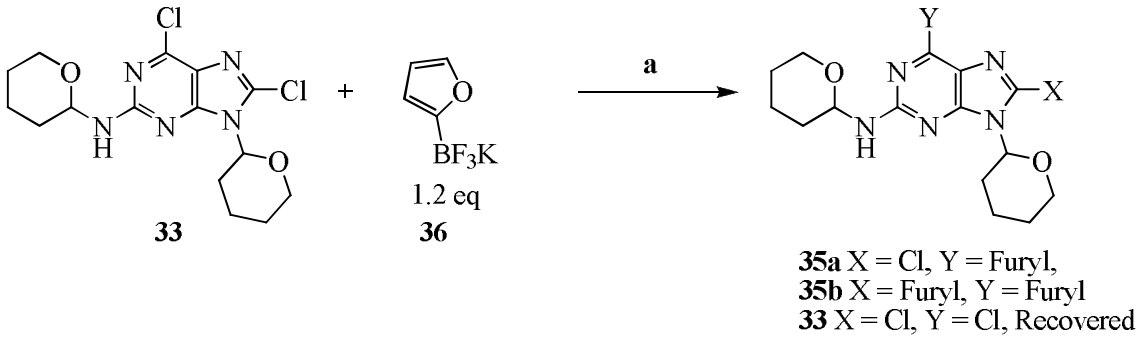
Table 2: Yields of products using Pd_2dba_3 (0.03 eq) and 2-furyl)3P (0.2 eq) as Catalyst .

Temperature (^oC)	**Compounds**	**Yield (%)**
50	**35a**	43
	35b	19
	33	13
60	**35a**	40
	35b	36
	33	0

Table 3: Yield of products using $pdCl_2.dppf.CH_2Cl_2$ (0.04eq) as catalyst.

Temperature (^oC)	**Compounds**	**Yield (%)**
50	**35a**	32
	35b	18
	33	26

Determined to improve the yield of these reactions a greener approach which employs the use of potassium organotrifluoroborates (**36)** as coupling partners for the Suzuki – Miyaura reaction[103] (Scheme 13) was attempted.



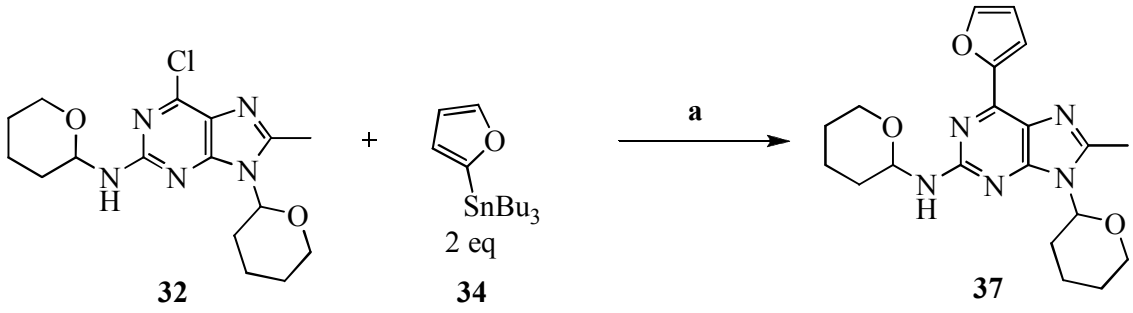
Scheme 13: Reagents and conditions: **a –** 1 mol % $Pd(OAc)_2$, 2 mol %Et_3N, 2 equiv Na_2CO_3 ethanol, 50^oC

Table 4: Comparison between Stille and Suzuki at 50 ^oC

Compound	**Stille (Yield %)**	**Suzuki (Yield %)**
35a	43	34
35b	19	20
33	23	12

However, no improvement was observed using Suzuki, but rather there was a little decrease in the yield of the product (Table 4). Moreover, it seems logical to use Stille coupling for this transformation, because at higher temperature, C-8 and / or C-6 halo purine are prone to nucleophilic attack by nucleophilic solvents like ethanol. Further optimisation of these conditions was not done.

2.1.4 6-(Furan-2-yl)-8-methyl-*N*,9-bis(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-2-amine. (37)



Scheme 14. Reagent and conditions. Pd-Cat, DMF, 90 ^{o}C

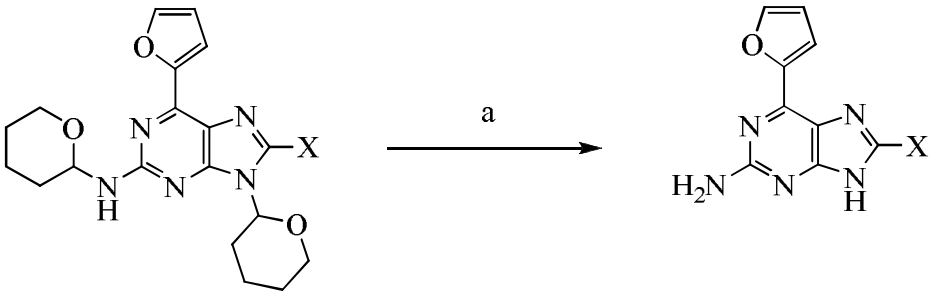
In this transformation (Scheme 14), a reactive catalyst $PdCl_2.dppf.CH_2Cl_2$ was required in order to achieve complete conversion. (Table 5), and compound **37** was isolated at good yield 68%.

Table 5

Catalysts	**Compounds**	**Yield (%)**
Pd_2dba_3 (0.03 eq) and 2-furyl)3P (0.2 eq)	**37**	35
	32	15
$pdCl_2.dppf.CH_2Cl_2$ (0.05 eq)	**37**	68
	32	0

2.1.5. 6-(Furan-2-yl)-8-methyl-9*H*-purin-2-amine (40), 8-Chloro-6-(furan-2-yl)-9*H*-purine-2-amine (38) and 6,8-di(furan-2-yl)-9*H*-purin-2-amine (39).

Compound **35a**, **35b** and **37** was deprotected to give compounds **38**, **39**, and **40** respectively (Scheme 15). This transformation resulted to the desired product. Longer reaction time was required for complete deprotection to compounds **38**, and **39** for reasons not understood.



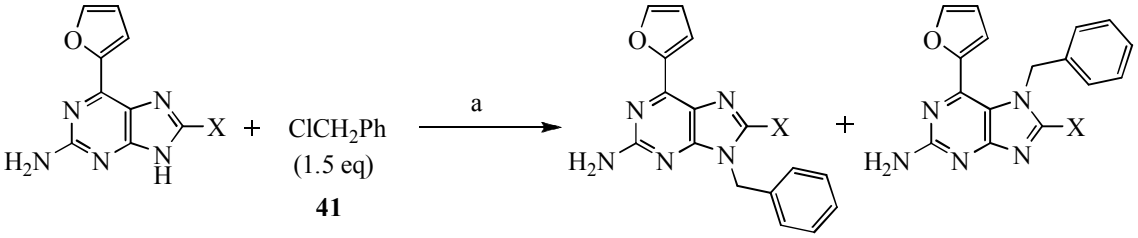
35a X = Cl
35b X = Furyl
37 X = CH_3

38 X = Cl, from **35a,** 88% 15hr
39 X = Furyl, from **35b,** 71% 15hr
40 X = CH_3, from **37,** 95% 1hr

Scheme 15: Reagents and condition: a – 9.6 M HCl, EtOH, rt.

2.1.6 9-Benzyl-8-chloro-6-(furan-2-yl)-9*H*-purin-2-amine (42a), 9-benzyl-6-(furan-2-yl)-8-methyl-9*H*-purin-2-amine (44a) and 9-benzyl-6,8-di(furan-2-yl)-9*H*-purin-2-amine (43a).

6-(Furan-2-yl)-8-methyl-9H-purin-2-amine(**40**), 8-chloro-6-(furan-2-yl)-9H-purine-2-amine(**38**) and 6,8-di(furan-2-yl)-9H-purin-2-amine(**39**) were *N*-alkylated with benzyl chloride (**41**) in the presence of potassium carbonate. (Scheme 16).



38 X = Cl
39 X = Furyl
40 X = CH_3

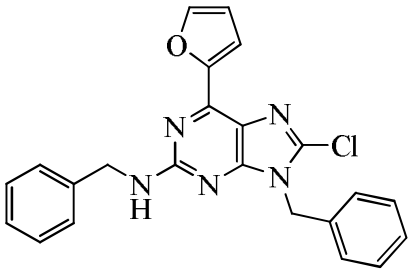
42a X = Cl, from **38,** 45%
43a X = Furyl, from **39,** 53%
44a X = CH_3, from **40,** 65%

42b X = Cl 25%
43b X = Furyl, 21%
44b X = CH_3, impure

Scheme 16. Reagents and condition. a - K_2CO_3 (3eq), DMF, rt

Both the *N*-9 and *N*-7 benzylated isomers were formed with the latter as the minor isomer but the *N*-7 isomers; **44b** was not isolated pure due to problems encountered during separation. Also a small amount of disubstitution **42c** was observed in the formation of compound **42a.** The ^{1}H NMR spectrum of the crude product showed the isomer distribution to be **42a** : **42b**; 2:1, **43a** : **43b**; 3:1, **44a** : **44b**; 2:1. The change in the ratio **43a** : **43b** could be attributed to the

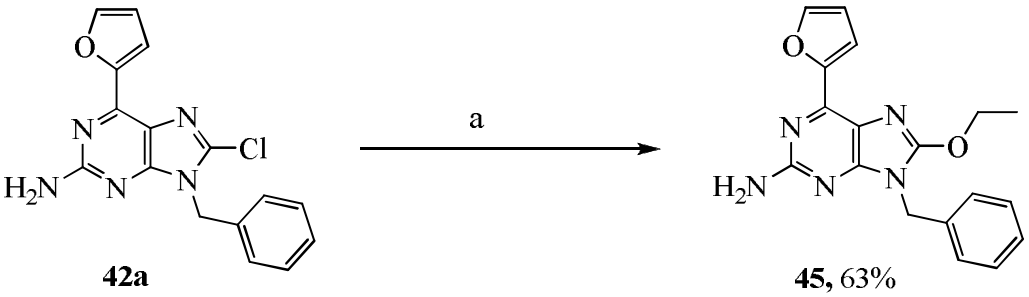
more bulky furyl groups in the 6 and 8 positions which could constitute sterical hindrance to the formation of *N*-7 isomer.



42c

2.1.7 9-Benzyl-8-ethoxy-6-(furan-2-yl)-9*H*-purin-2-amine (45).

By using 8-halopurines relatively easy nucleophilic attack takes place at 8-position with a wide range of nucleophiles such as alkoxide, sulphides, amines, azide, cyanide, and malonate and related carbanions.[90] Compound **42a** was subjected to nucleophilic substitution reaction with sodium ethoxide to afford the introduction of ethoxy group in this position (Scheme 17). In this reaction, desired product **45** was formed in 63% yield.



42a **45,** 63%

Scheme 17: a – NaOEt in EtOH (0.216 M), 15 h, rt

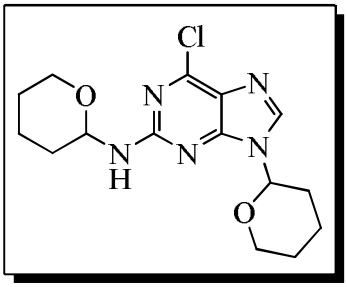
3.0 Conclusion.

All the three target compounds were successfully synthesised and have been submitted for biological testing together with the N-alkylated dicoupled Product (**43a**). In this study we want to apply existing models for the ARs and perform docking studies of ligands with selective affinity for the various ligands with the purpose of designing more potent and selective ligands. These studies will be carried out by the groups of Prof. Christa Müller and collaborators especially Dr. Astrid Maaß at the Fraunhofer Institute, SCAI, St. Augustin Germany.

4.0 EXPERIMENTAL

The ^{1}H NMR spectra were recorded at 300MHz with Bruker DRX 300 or at 200 MHz with Bruker DPX 200 intrument, The ^{13}C NMR spectra were recorded at 75MHz using Bruker DRX 300 instrument. Mass spectra were recorded by electron ionization at 70eV ionizing voltage and are presented as m/z (% relative intensity). The chemical shifts (δ) are given in ppm. THF and DMF were obtained from automated solvent drying system. Diisopropylamine was distilled from CaH_2. Melting points were determined with a Büchi melting point B-545 apparatus and are uncorrected. Elemental analysis were performed at School of Chemistry, University of Birmingham, Edgbaston, Birmingham. All other reagents were commercially available and used as received.

4.1 Synthesis of 6-Chloro-*N*,9-bis(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-2-amine (31).



31

To a mixture of 6-chloro-9*H*-purin-2-amine **(29)** (170 mg, 1 mmol) in DMF 10 mL, 2M HCl in DMF (50 µl, 0.1 mmol) was added and the mixture was stirred at 60 ^{o}C for 5 min and then 3,4-dihydro-2*H*-pyran **(30)** (0.45 mL, 5 mmol) was added dropwise. The stirring was continued at 60 ^{o}C for 2h and the solvent was evaporated. A column chromatography (silica gel, EtOAc: hexane 3:1) of the crude, afforded the protected derivative.

Yield 216mg (63%) Colourless Powder

M.P. 170 – 172 ^{o}C

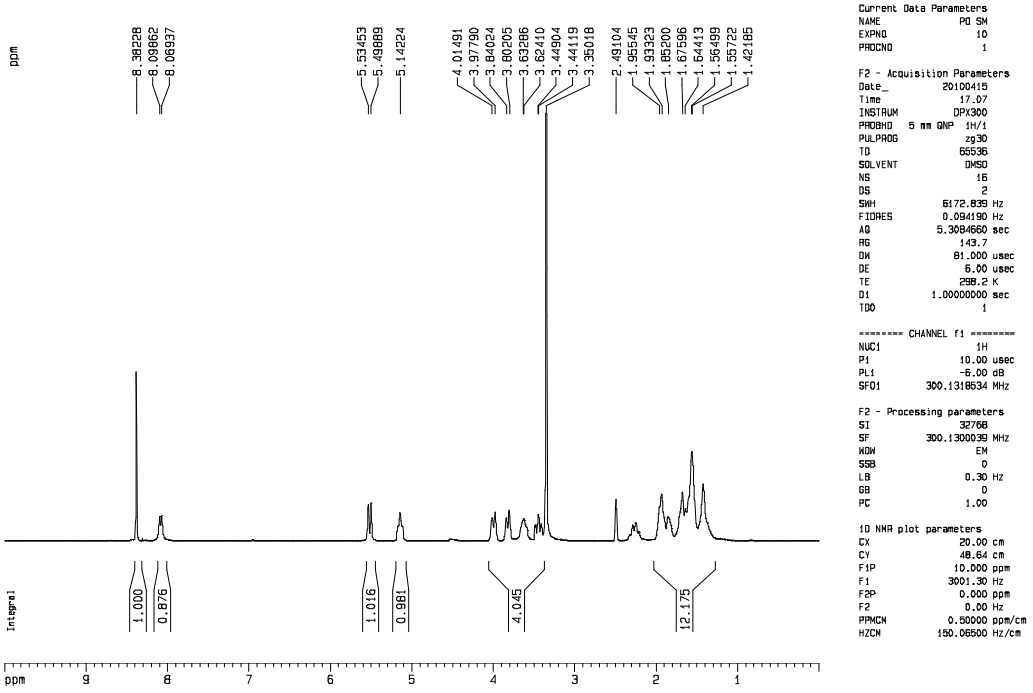
^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.42 – 1.96 (m, 12H, CH_2 in THP), 3.44 – 4.01 (m, 4H, OCH_2 in THP), 5.14 (br s, 1H, THP), 5.52 (d, J = 10.7Hz, 1H, THP), 8.08 (d, J = 8.8 Hz, 1H, NH), 8.38 (s, 1H, H-8).

^{13}C NMR (DMSO-d_6, 75 MHz): δ 23.26, 25.32 , 25.81, 30.39, 30.48, 31.07 (CH_2 in THP), 66.42 (OCH_2 in THP), 68.55 (OCH_2 in THP), 80.87 (CH in THP), 81.94 (CH in THP), 125.05 (C-5), 142.68 (C-8), 150.42 (C-6), 154.04 (C-4), 158.49 (C-2)

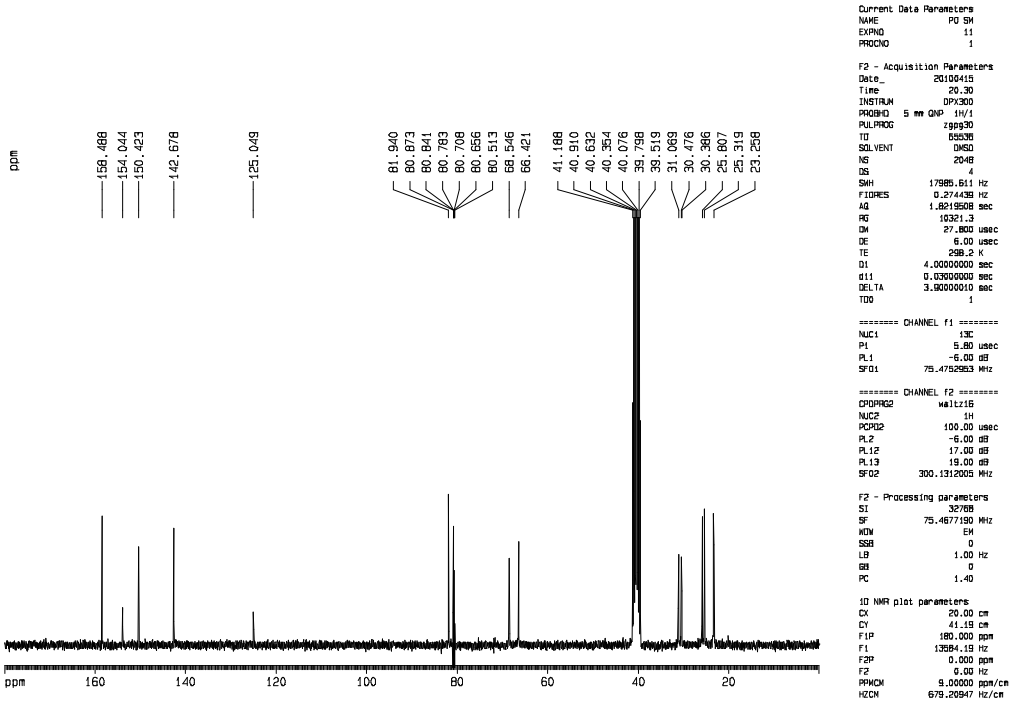
MS EI, m/z (rel. %)**:** 337 (8, M^+), 253(17), 169 (100), 85 (61)

HR-MS: Found 337.129800, calculated value for $C_{15}H_{20}ClN_5O_2$ 337.1382

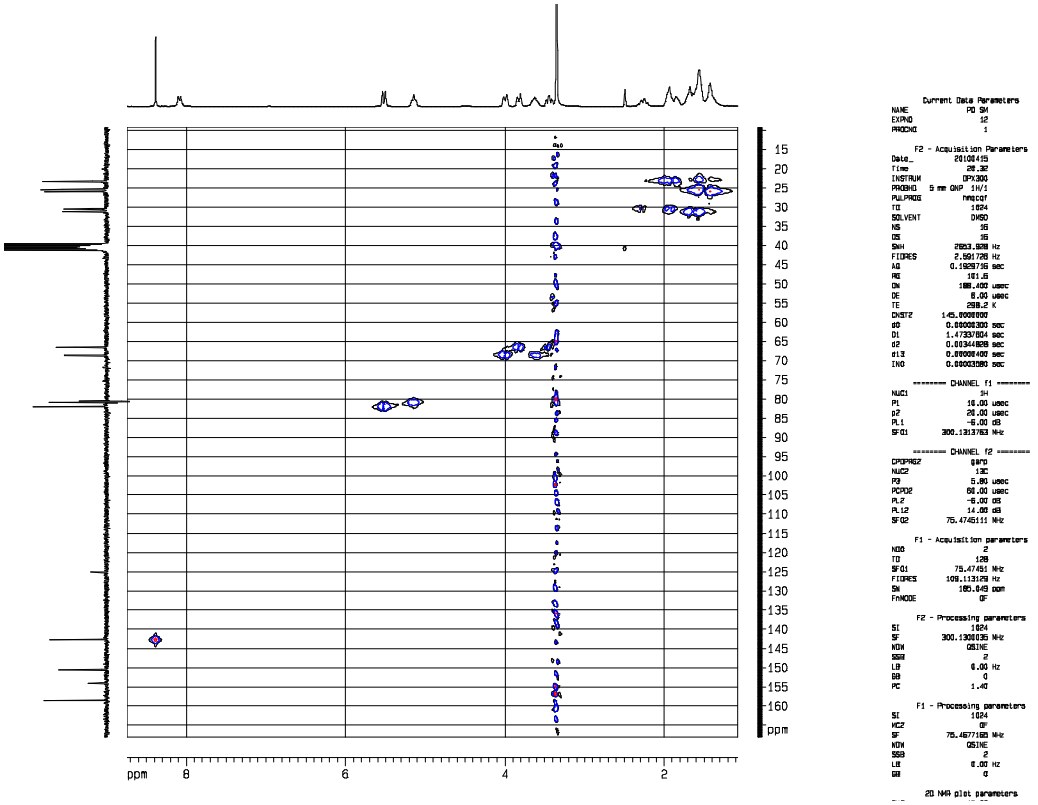
.



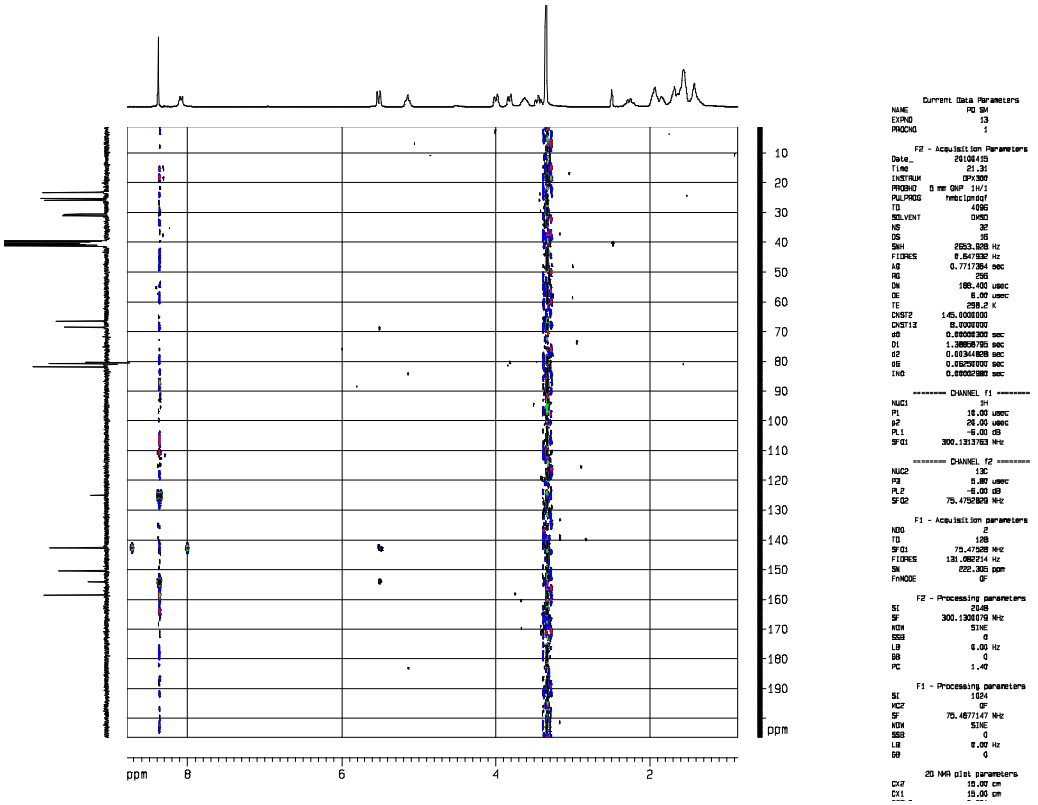
Spectrum 1. [1]H NMR of 6-Chloro-*N*,9-bis(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-2-amine **(31)**.



Spectrum 2. [13]C NMR of 6-Chloro-*N*,9-bis(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-2-amine **(31)**.

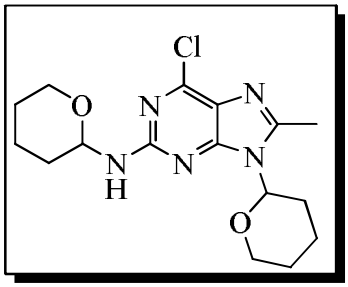


Spectrum 3. HMQC of 6-Chloro-*N*,9-bis(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-2-amine **(31)**.



Spectrum 4. HMBC of 6-Chloro-*N*,9-bis(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-2-amine **(31)**.

4.2 Synthesis of 6-Chloro-8-methyl-*N*,9-bis(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-2-amine (32).



32

A solution of diisopropylamine (2.2 mmol 0.3 ml) in dry THF (4 mL) was stired at -78^{o}C under N_2. *n*-BuLi (1.25 mL, 2 mmol, 1.6 M in hexane) was added dropwise. After stiring for 20 min, a solution of 6-chloro-*N*,9-bis(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-2-amine **(31)** (1 mmol, 0.338 g) in THF (4 mL) was added. After additional stirring for 1 h at -78^{o}C, iodomethane (0.62 mL, 10 mmol) was added dropwise and the resulting mixture was stirred at -78^{o}C for 3.5 h, gradually warmed to ambient temperature over 2 h and stirred at ambient temperature for 15 h. **work up:** Sat aqNH_4Cl (30 mL) was added and the mixture was extracted with EtOAc (2 x 50 mL). the combined organic extracts were washed with brine (40 mL), dried ($MgSO_4$) and evaporated *in vacuo*. The product was purified by flash chromatography on silica gel eluting with a EtOAc/hexane mixture (5:1) .

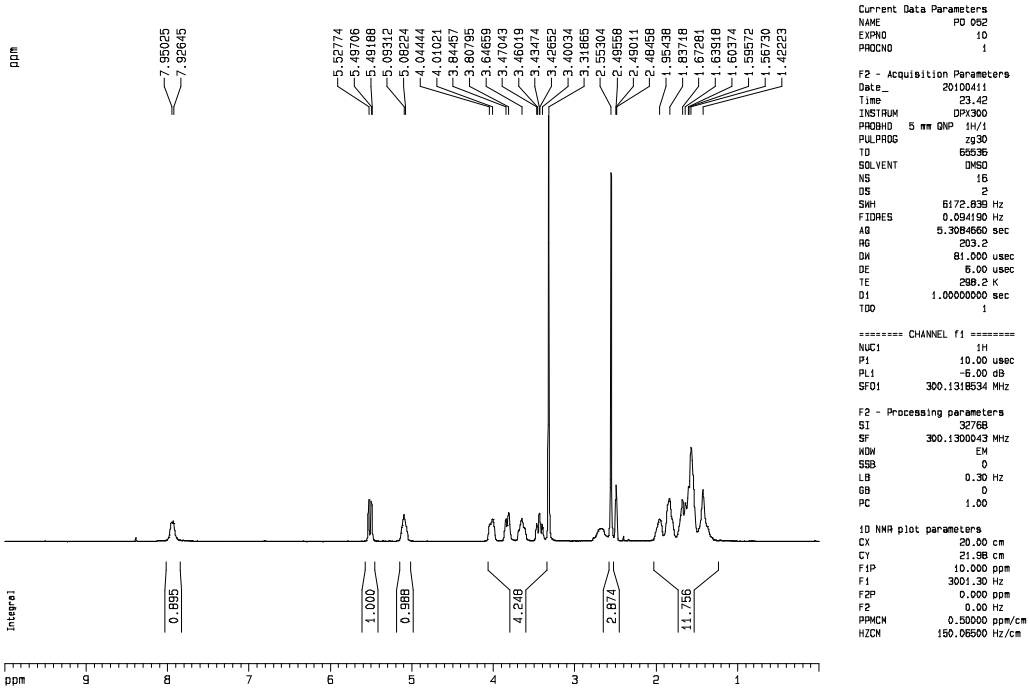
Yield 310 mg, (90%), Pale yellow solid. M.p 184 – 186 ^{o}C,

^{1}H NMR (DMSO- d_6, 300 MHz): δ 1.42 – 1.95 (m, 12H, THP), 2.55 (s, 3H, CH_3), 3.40 – 4.04 (m, 4H, THP), 5.09 (br s, 1H, THP), 5.49 – 5.53 (m, 1H, THP), 7.94 (d, *J* = 7.1 Hz, 1H, NH).

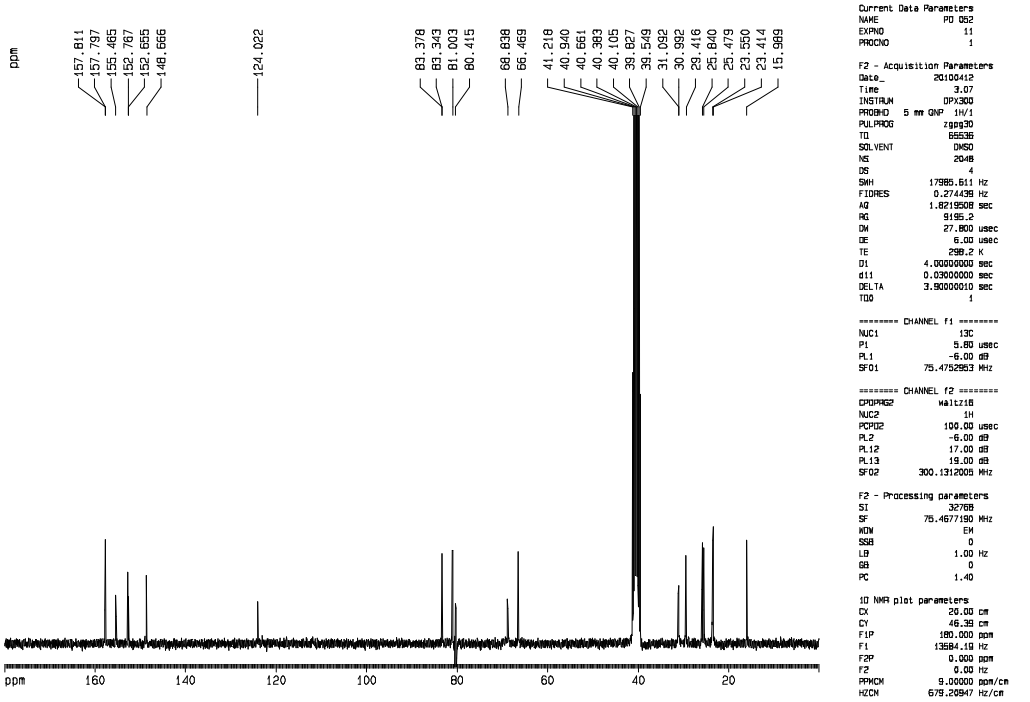
^{13}C NMR (DMSO- d_6, 75 MHz): δ 15.99 (CH_3), 23.41, 23.55 , 25.48, 25.84, 29.42, 30.99 (CH_2 in THP), 66.47 (OCH_2 in THP), 68.84 (OCH_2 in THP), 81.00 (CH in THP), 83.36 (CH in THP), 124.02 (C-5), 148.67 (C-6), 152.71 (C-8), 155.47 (C-4), 157.80 (C-2).

MS EI m/z (rel. %)**:** 353/351 (3/9, M^+), 267 (15) , 183/185 (37/100), 148 (8)

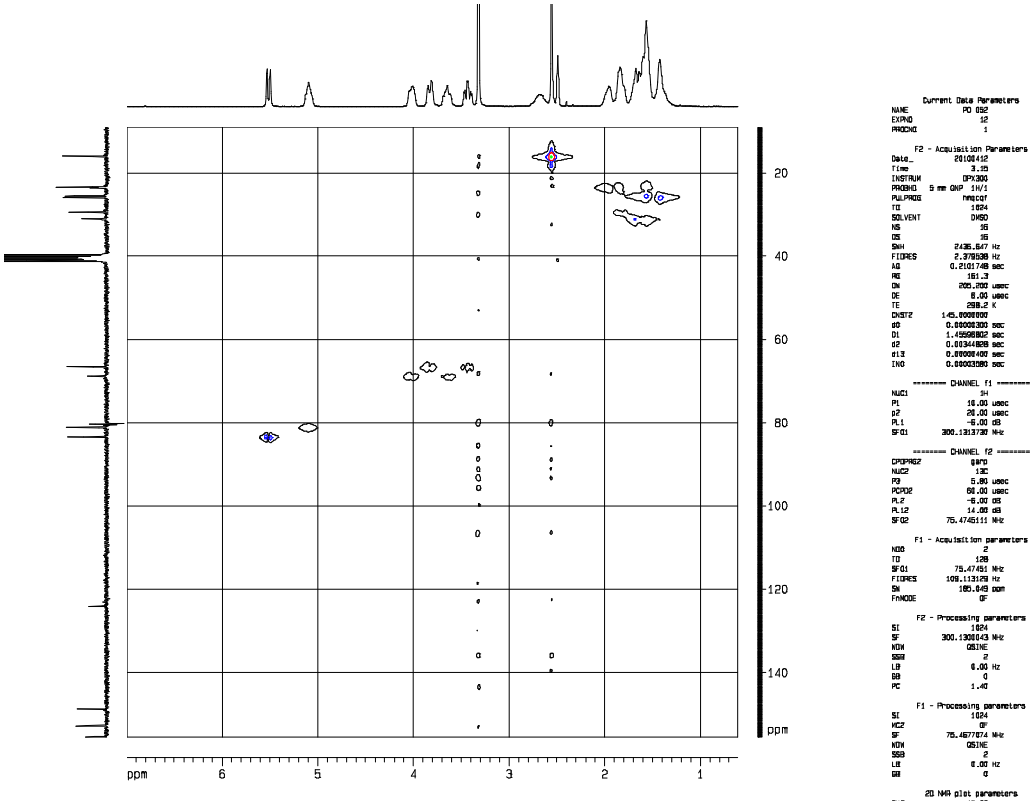
HR-MS: Found 351.145160, calculated value for $C_{16}H_{22}ClN_50_2$ 351.1462.



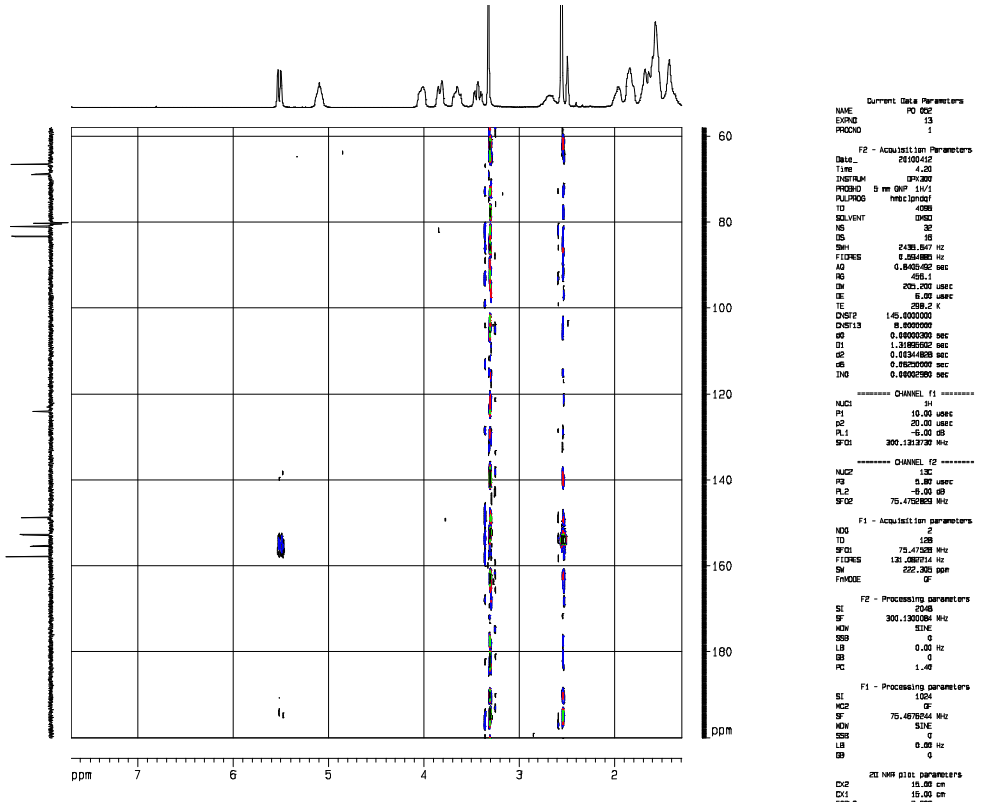
Spectrum 5. ^{1}H NMR of 6-Chloro-8-methyl-*N*,9-bis(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-2-amine **(32)**.



Spectrum 6. ^{13}C NMR 6-Chloro-8-methyl-*N*,9-bis(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-2-amine **(32)**.

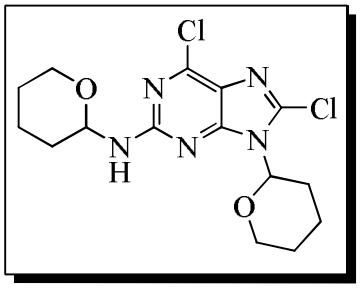


Spectrum 7. HMQC of 6-Chloro-8-methyl-*N*,9-bis(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-2-amine**(32)**.



Spectrum 8. HMBC of 6-Chloro-8-methyl-*N*,9-bis(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-2-amine **(32)**.

4.3 Synthesis of 6,8-dichloro-*N*,9-bis(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-2-amine (33).



33

A solution of diisopropylamine (0.81 mL, 5.94 mmol) in dry THF (10.8mL) was stired at -78^{o}C under N_2. *n*-BuLi (5.4 mL, 5.4 mmol, 1.0 M in hexane) was added dropwise. After stirring for 20 min, a solution of 6-chloro-*N*,9-bis(tetrahydro-2*H*-pyran-2-yl)-9H-purin-2-amine **(31)** (1 g, 2.7 mmol) in THF (10.8mL) was added. After additional stirring for 1 h at -78^{o}C, a solution of hexachloroethane (1278 mg, 5.4 mmol) in THF (10.8 mL) was added dropwise and the resulting mixture was stirred at -78^{o}C for 2 h, and 10 min without cooling, sat aq NH_4Cl (81 mL) was added and the resulting mixture was extracted with EtOAc (2 x 135 mL). The combined organic extracts was washed with brine (108 mL), dried with $MgSO_4$ and evaporated *in vacuo*. Product was purified by flash chromatography on silica gel eluting with EtOAc/hexane mixture (1:1)

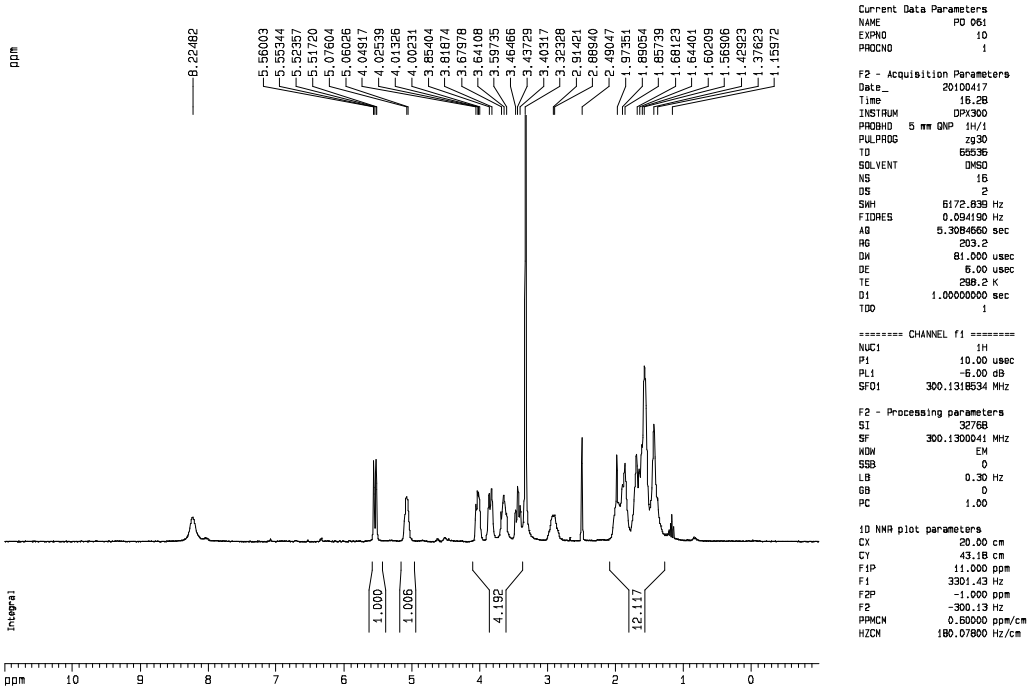
Yield 0.91 g, (91%) yellow powder. M.p 140 – 142 ^{o}C,

^{1}H NMR (DMSO- d_6, 300 MHz): δ 1.56 – 1.97 (m, 12H, THP), 3.40 – 4.05 (m, 4H, THP), 5.07 (br s, 1H, THP), 5.52 – 5.56 (m, 1H, THP), 8.23 (br s, 1H, NH).

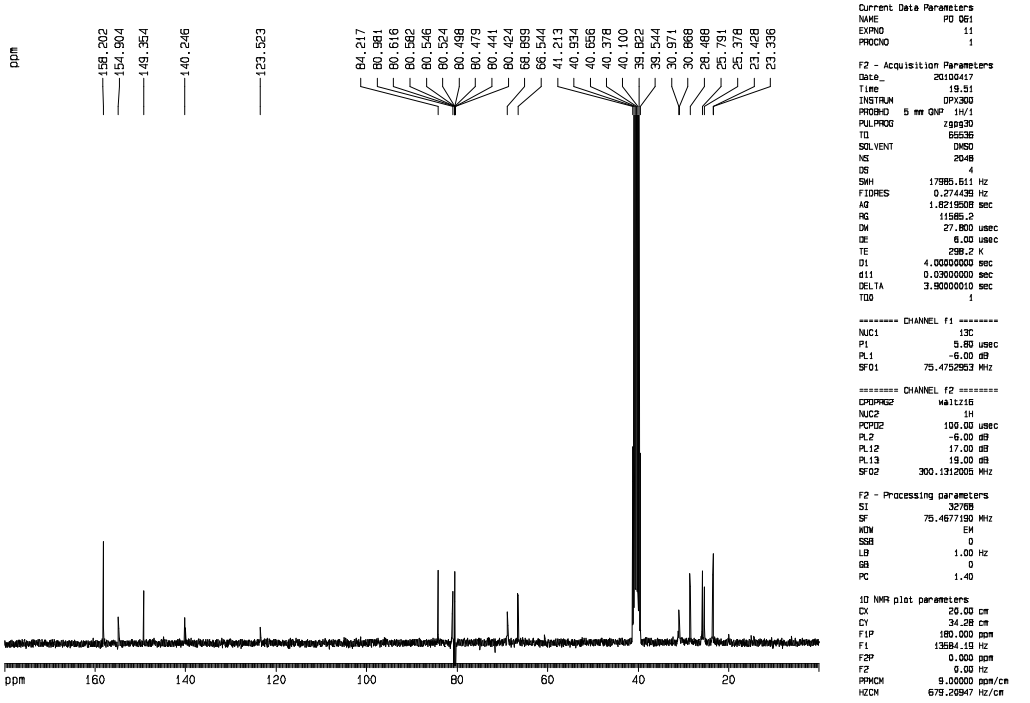
^{13}C NMR (DMSO- d_6, 75 MHz): δ 23.34, 23.43, 25.38, 25.79, 28.49, 30.87 (CH_2 in THP), 66.54 (OCH_2 in THP), 68.90 (OCH_2 in THP), 80.98 (CH in THP), 84.22 (CH in THP), 123.52 (C-5), 140.25 (C-4), 149.35 (C-6), 154.86 (C-8), 158.20 (C-2).

MS (EI) m/z (rel. %)**:** 375/373/371 (2/8/13, M^+), 291/289/287 (5/30/45), 207/205/203 (18/79/100), 168 (13)

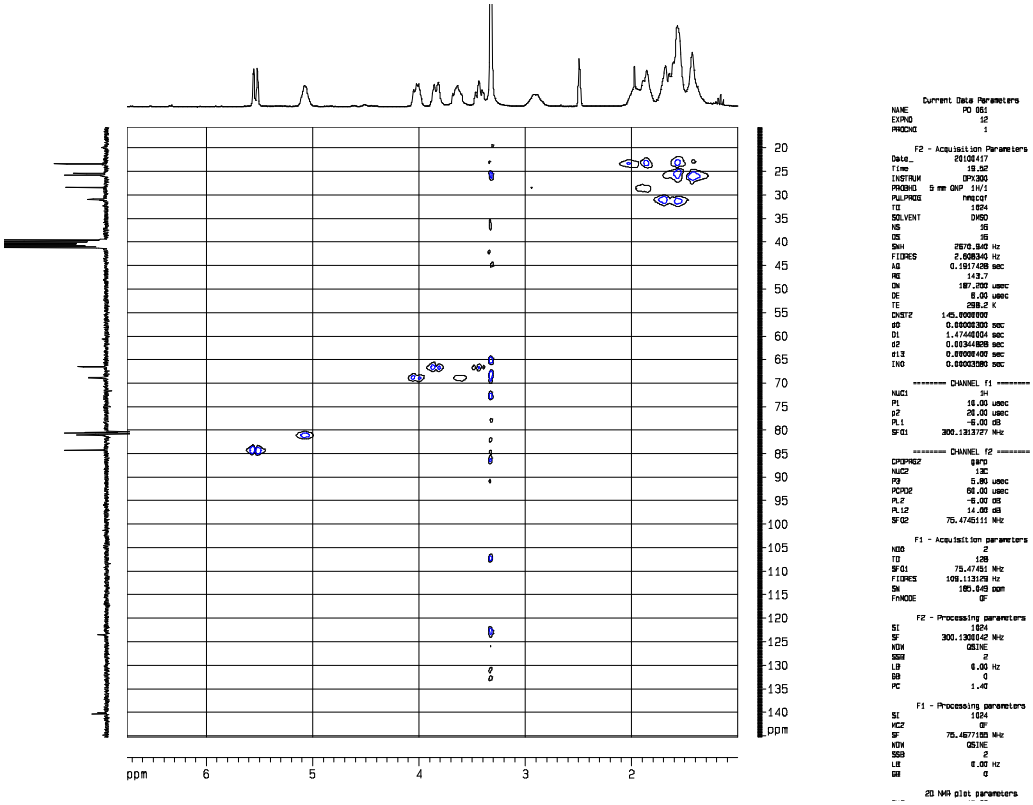
HR-MS: Found 371.091962, caculated value for $C_{15}H_{19}Cl_2N_5O_2$ 371.0916



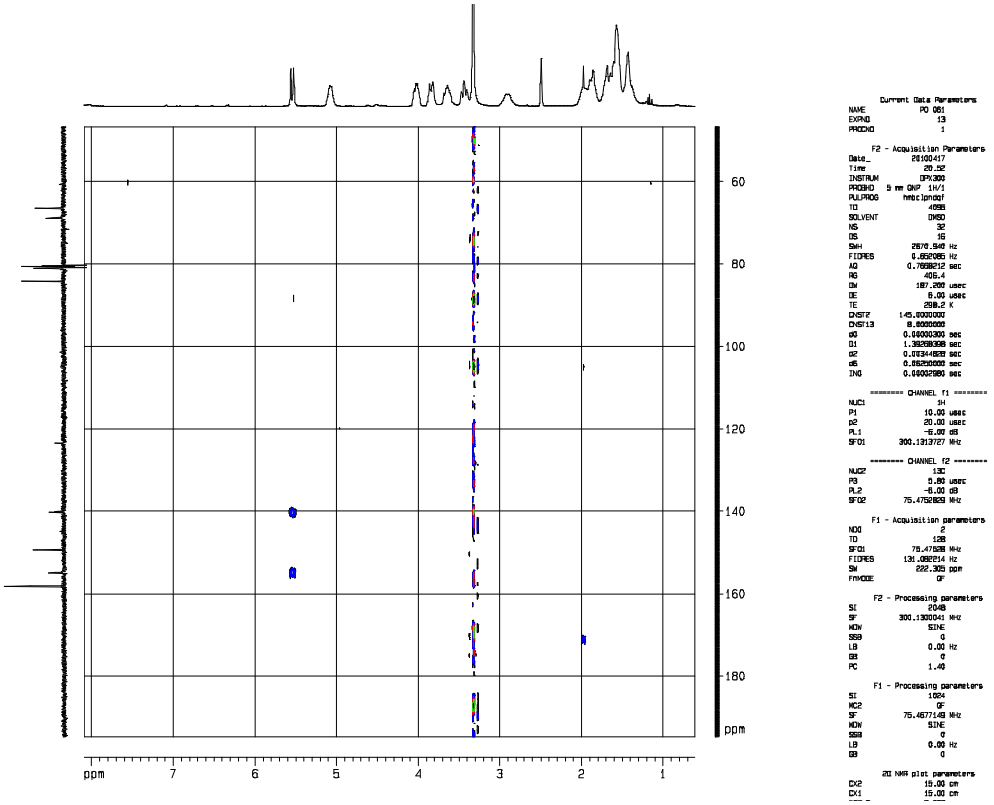
Spectrum 9. ^{1}H NMR of 6,8-dichloro-*N*,9-bis(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-2-amine **(33)**.



Spectrum 10. ^{13}C NMR of 6,8-dichloro-*N*,9-bis(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-2-amine **(33)**.

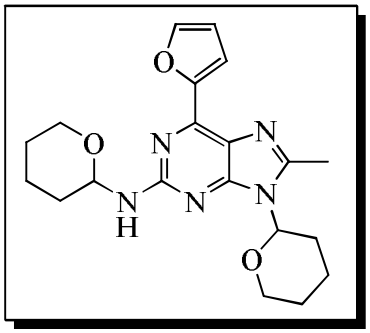


Spectrum 11. HMQC of 6,8-dichloro-*N*,9-bis(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-2-amine **(33)**.



Spectrum 12. HMBC of 6,8-dichloro-*N*,9-bis(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-2-amine **(33)**.

4.4 Synthesis of 6-(Furan-2-yl)-8-methyl-*N*,9-bis(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-2-amine (37).



37

2-furyl (tributyl) tin **(34)** (0.53 mL, 1.66 mmol) was added to a solution of 6-chloro-8-methyl-*N*,9-bis(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-2-amine **(32)** (290 mg, 0.83 mmol) and $pdCl_2.dppf.CH_2Cl_2$ (52 mg, 0.04 mmol) in DMF (9 mL). The resulting mixture was stirred at 90°C under N_2 for 16 h, cooled and evaporated *in vacuo*. Crude reaction mixture was partitioned between Acetonitrile (30 mL) and hexane (10 mL), repeated washings of MeCN layer with hexane allowed removal of lipophilic tributyl tin chloride. The product was purified by flash chromatography on silica gel eluting with EtOAc/hexane (1:1).

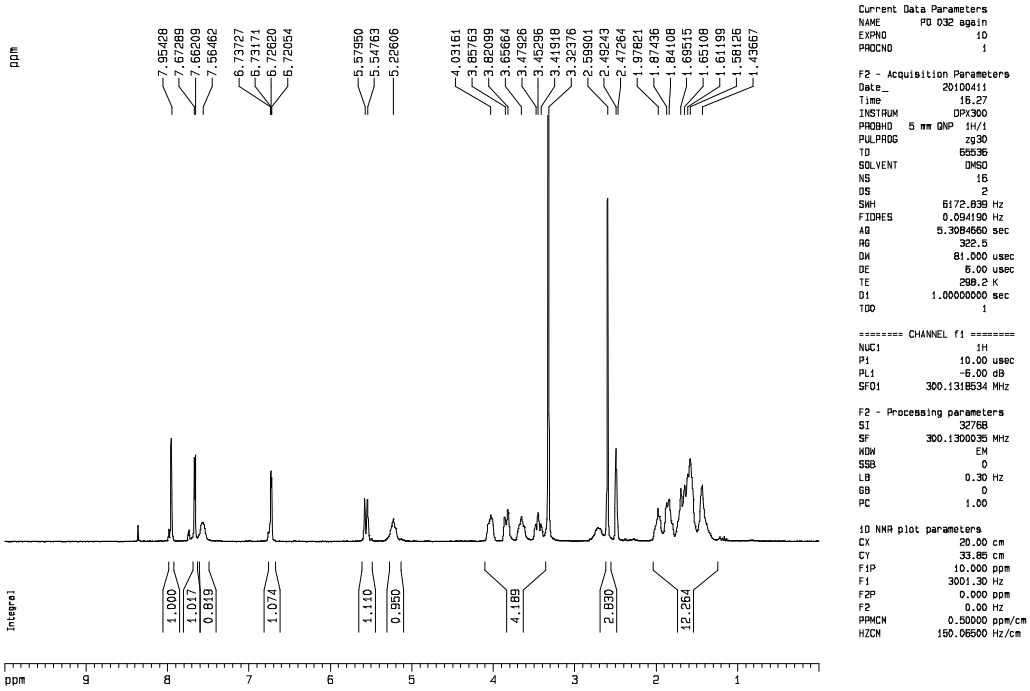
Yield 215 mg, (68 %), colourless powder. M.p 155 – 157°C,

^{1}H NMR (DMSO- d_6, 300 MHz): δ 1.44 – 1.98 (m, 12H, THP), 2.60 (s, 3H, CH_3), 3.42 – 4.03 (m, 4H, THP), 5.23 (br s, 1H, THP), 5.55 – 5.58 (m, 1H, THP), 6.73 (dd, *J* = 3.3, 1.7 Hz, 1H, H-4 in furyl), 7.57 (br, s, 1H, NH), 7.67 (d, *J* = 3.24 Hz, 1H, H-3 in furyl), 7.95 (s, H-5 in furyl).

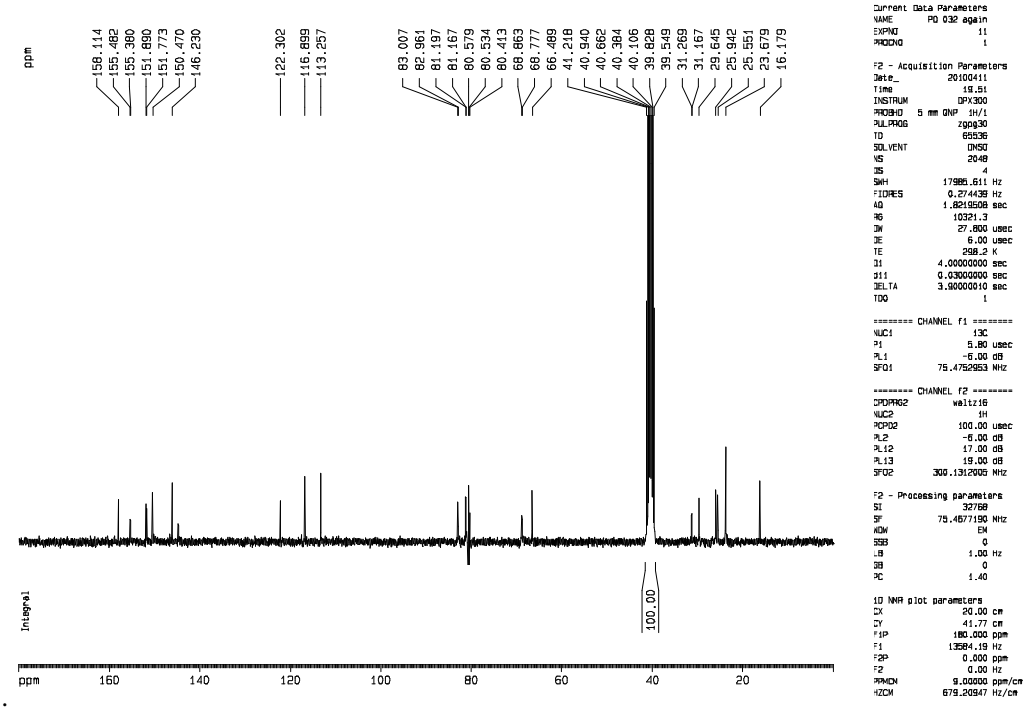
^{13}C NMR (DMSO- d_6, 75 MHz): δ 16.18 (CH_3) 23.68, 25.55, 25.94, 29.65, 31.17, 31.27 (CH_2 in furyl), 66.49 (OCH_2 in THP), 68.78 (OCH_2 in THP), 81.20 (CH in THP), 82.96 (CH in THP), 113.26 (C-4 in furyl), 116.90 (C-3 in furyl), 122.30 (C-5), 144.74 (C-6), 146.23 (C-5 in furyl), 150.47 (C-2 in furyl) 151.77 (C-8), 155.38 (C-4), 158.11 (C-2),

MS (EI) m/z (rel %)**:** 383 (10, M^+), 299 (11), 215 (100), 199 (5), 147 (2)

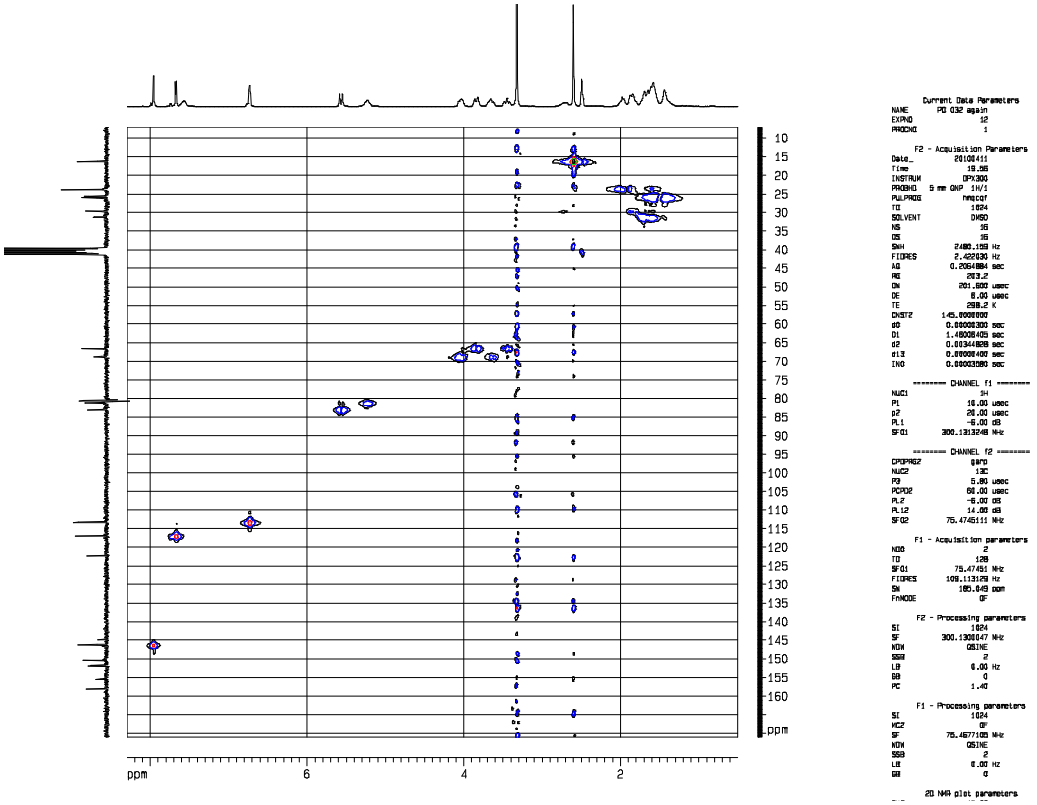
HR-MS: Found 383.2019, caculated value for $C_{20}H_{25}N_5O_3$ 383.1957



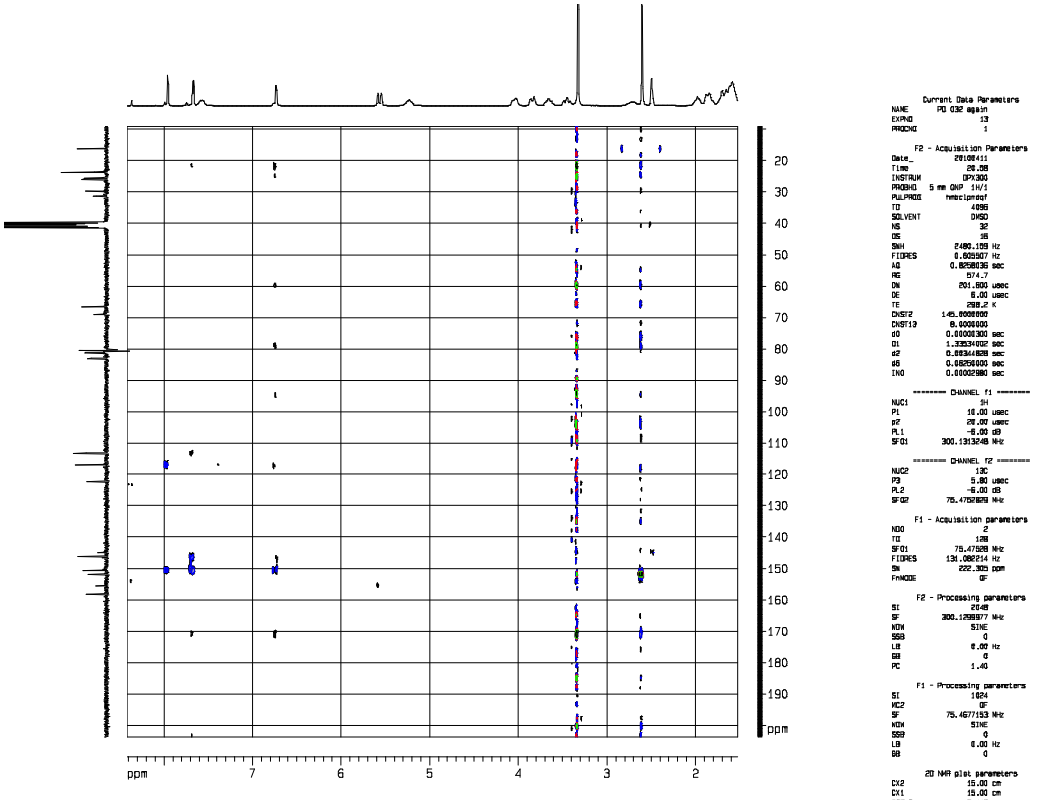
Spectrum 13. ^{1}H NMR of 6-(Furan-2-yl)-8-methyl-*N*,9-bis(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-2-amine **(37).**



Spectrum 14. ^{13}C NMR of 6-(Furan-2-yl)-8-methyl-*N*,9-bis(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-2-amine **(37).**

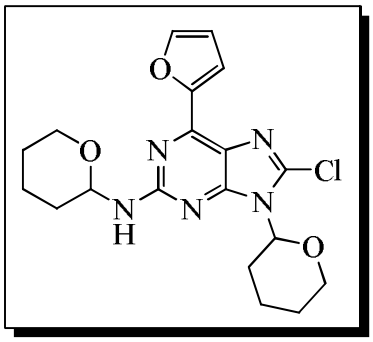


Spectrum 15. HMQC of 6-(Furan-2-yl)-8-methyl-*N*,9-bis(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-2-amine **(37).**

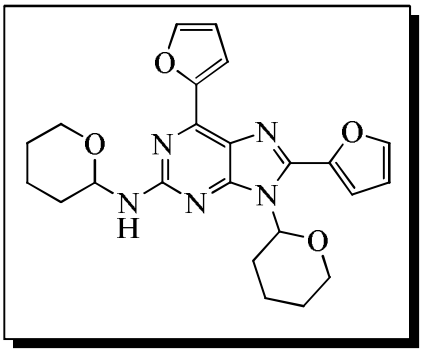


Spectrum 16. HMBC of 6-(Furan-2-yl)-8-methyl-*N*,9-bis(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-2-amine **(37).**

4.5 Synthesis of 8-Chloro-6-(Furan-2-yl)-*N*,9-bis(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-2-amine (35a) and 6,8-di(furan-2-yl)-*N*,9-bis(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-2-amine (35b).



35a



35b

A mixture of tris (diphenylmethylideneacetone) – dipalladium chloroform adduct (66 mg, 0.062 mmol) and tri (2-furyl) phosphine (105 mg, 0.45 mmol) in DMF (25 mL) was stirred at ambient temperature under N_2 for 15 min, before 6,8-dichloro-*N*,9-bis(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-2-amine **(33)** (780 mg, 2.1 mmol) and 2-furyl (tributyl) tin **(34)** (0.8 mL, 2.52 mmol) were added. The resulting mixture was stirred for 18 h at 50^{o}C and evaporated. The residue was dissolved in acetonitrile (80 mL) and washed with hexane (15 X 25 mL). The acetonitrile layer was evaporated and the products were isolated by flash chromatography on silica eluting with EtOAc/hexane (1:2) followed by EtOAc/hexane (1:1).

4.6 Synthesis of 8-Chloro-6-(Furan-2-yl)-*N*,9-bis(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-2-amine (35a).

Yield 360 mg, (43 %), Colourless powder. M.p 162 – 164 ^{o}C,

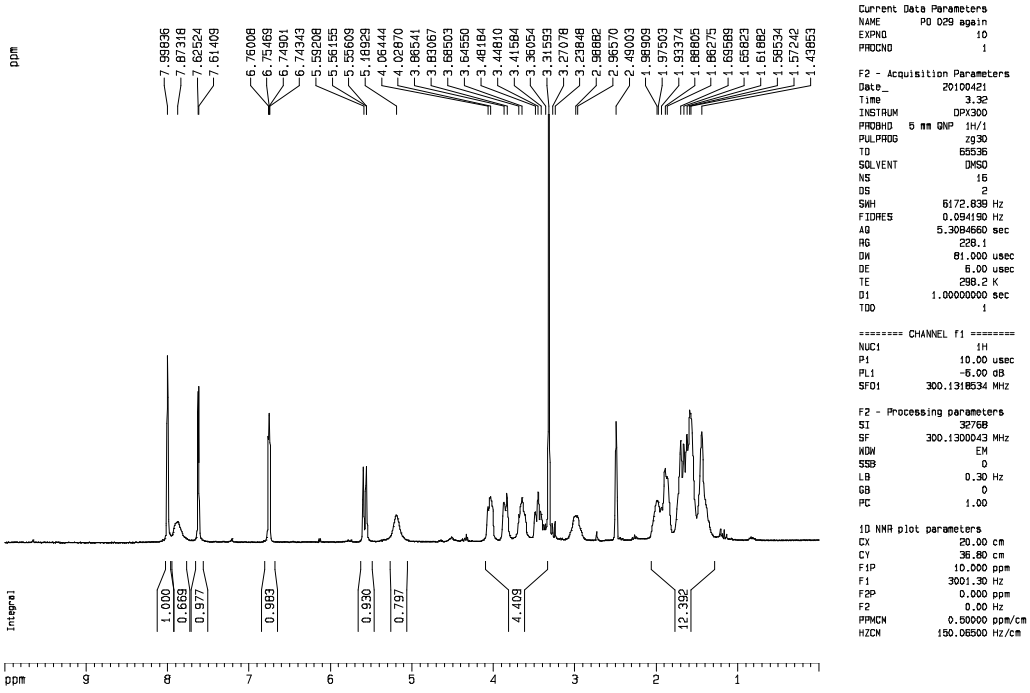
^{1}H NMR (DMSO- d_6, 300 MHz): δ 1.44 – 1.99 (m, 12H, THP), 3.42 – 4.06 (m, 4H, THP), 5.19 (br s, 1H, THP), 5.56 – 5.59 (m, 1H, THP), 6.75 (dd, J = 3.3, 1.7 Hz, 1H, H-4 in furyl), 7.62 (d, J = 3.4 Hz, 1H, H-3 in furyl), 7.87 (br, s, 1H, NH), 8.00 (s, H-5 in furyl).

^{13}C NMR (DMSO- d_6, 75 MHz): δ 23.60, 25.44, 25.89, 28.63, 31.01, 31.13 (CH_2 in THP), 66.54 (OCH_2 in THP), 68.90 (OCH_2 in THP), 81.13 (CH in THP), 83.85 (CH in THP), 113.50

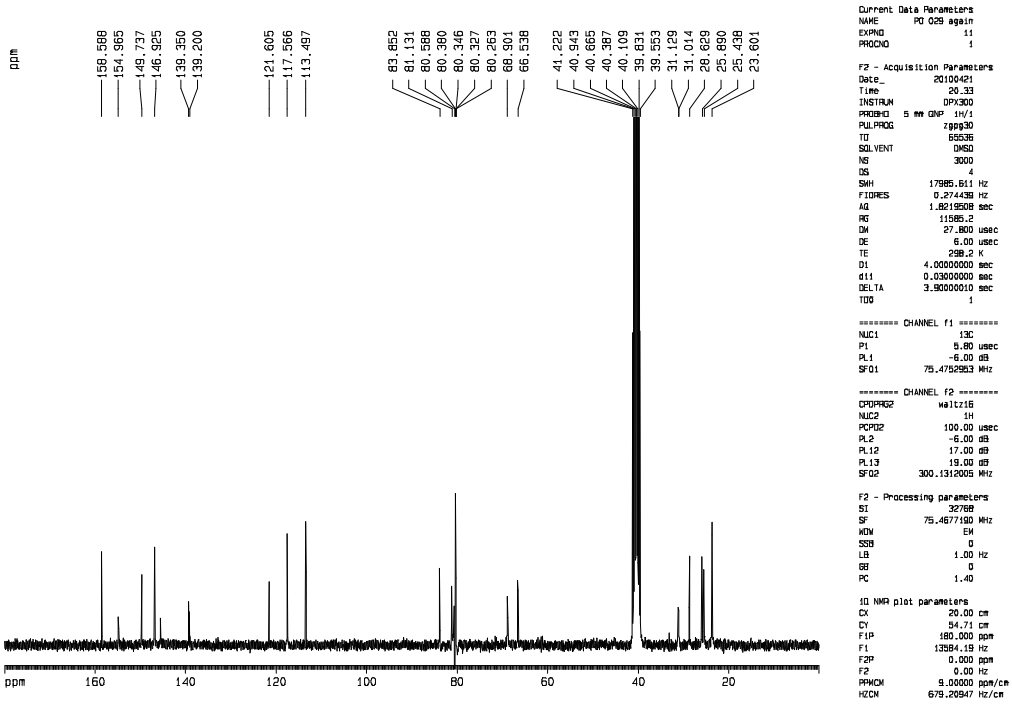
(C-4 in furyl), 117.57 (C-3 in furyl), 121.61 (C-5), 139.20 (C-4), 145.56 (C-6), 146.92 (C-5 in furyl), 149.74 (C-2 in furyl), 154.97 (C-8), 158.59 (C-2),

MS (EI) m/z (rel. %)**:** 405/403 (2/6, M^+), 321/319 (8/24), 237/235 (36/100), 85 (13).

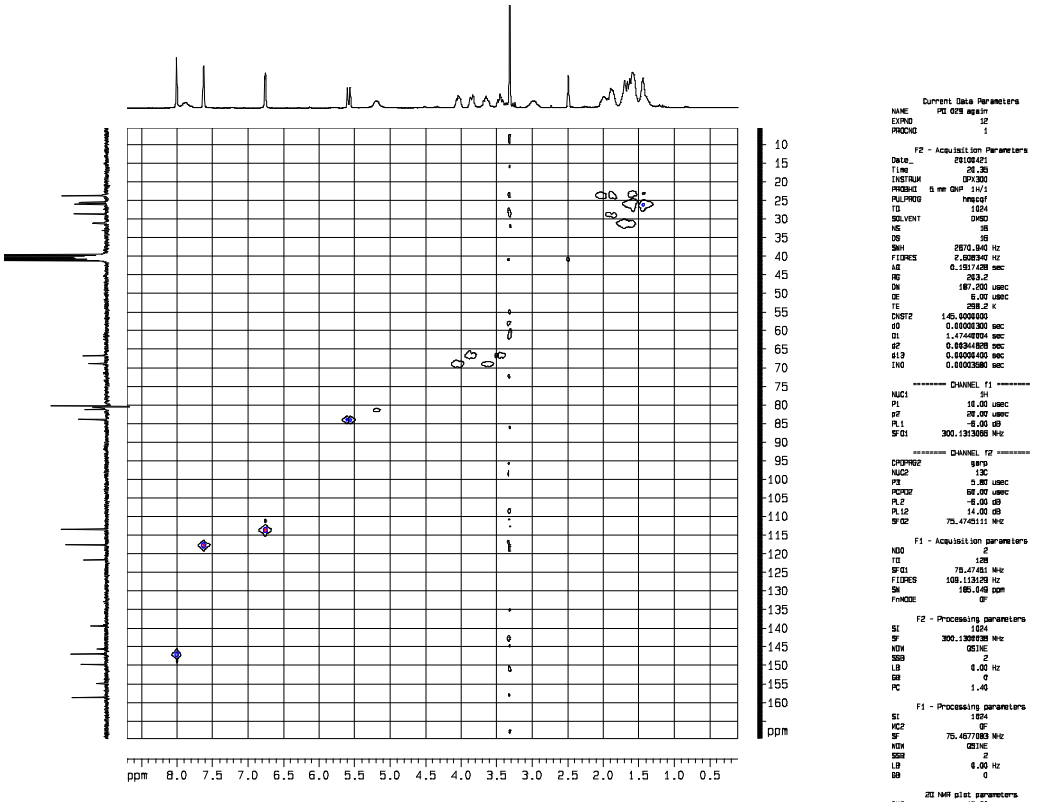
HR-MS: Found 403.141679, calculated value for $C_{20}H_{25}N_5O_3$ 403.1411.



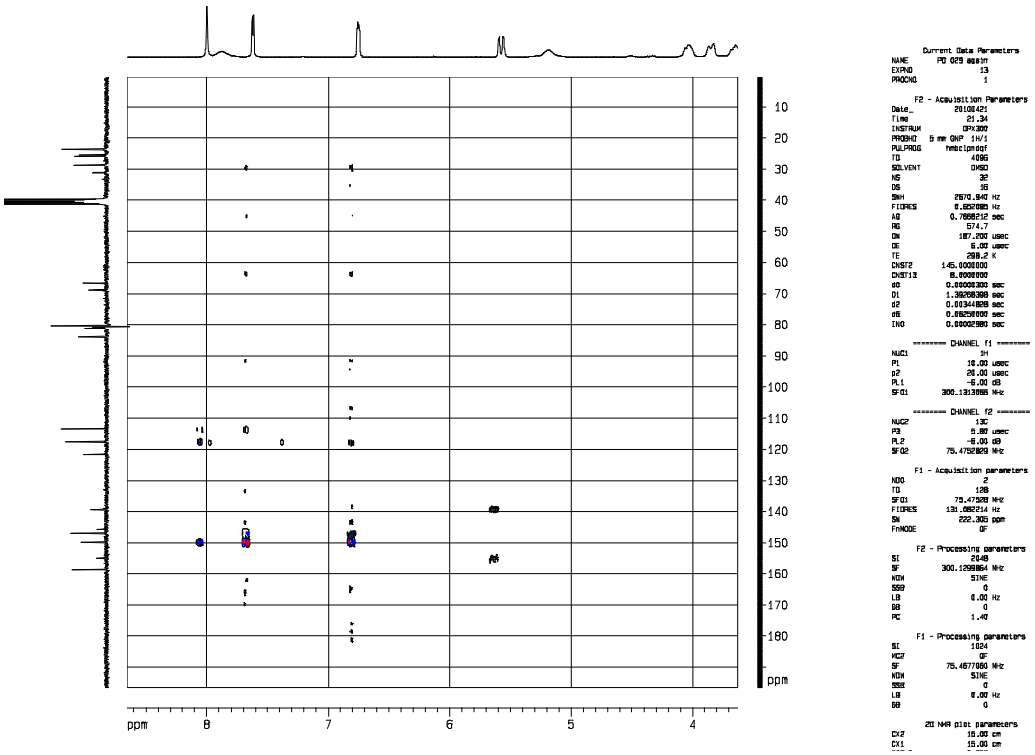
Spectrum 17. ^{1}H NMR of 8-Chloro-6-(Furan-2-yl)-*N*,9-bis(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-2-amine **(35a).**



Spectrum 18. ^{13}C NMR of 8-Chloro-6-(Furan-2-yl)-*N*,9-bis(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-2-amine **(35a).**



Spectrum 19. HMQC of 8-Chloro-6-(Furan-2-yl)-*N*,9-bis(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-2-amine **(35a).**



Spectrum 20. HMBC of 8-Chloro-6-(Furan-2-yl)-*N*,9-bis(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-2-amine **(35a).**

4.7 Synthesis of 6,8-di(furan-2-yl)-*N*,9-bis(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-2-amine (35b).

Yield 200 mg, (19 %), dark-yellowish powder. M.p 154 -157 ^{o}C

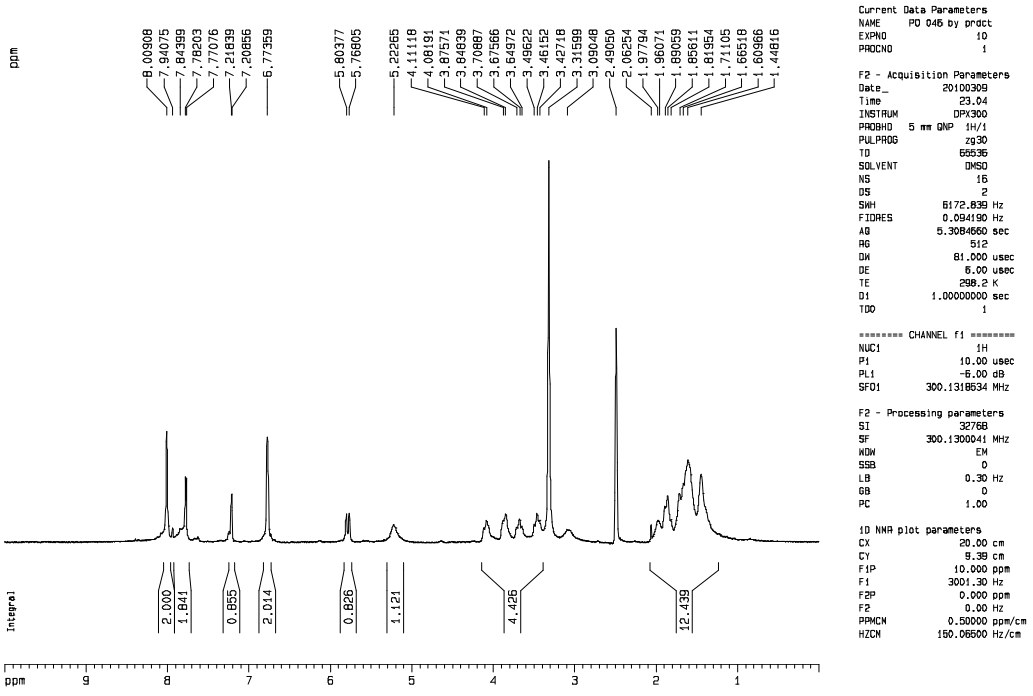
^{1}H NMR (DMSO- d_6, 300 MHz): δ 1.45 – 2.06 (m, 12H, THP), 3.43 – 4.11 (m, 4H, THP), 5.22 (br s, 1H, THP), 5.79 (d, J = 10.7Hz, 1H, THP), 6.77 (br s, 2H, H-4 in furyl), 7.21 (d, J = 3.0 Hz, 1H, H-3 in furyl), 7.78(d, J = 3.4, 1H, H-3 in furyl), 7.84 (br, 1H, NH), 8.01 (s, 2H H-5 in furyl).

^{13}C NMR (DMSO- d_6, 75 MHz): δ 23.69, 23.79, 25.46, 25.94, 28.74, 31.19 (CH_2 in THP), 66.57 (OCH_2 in THP), 68.71 (OCH_2 in THP), 81.31 (CH in THP), 84.16 (CH in THP), 113.08 (C-4 in furyl), 113.47 (C-4 in furyl) 114.96 (C-3 in furyl), 117.76 (C-3 in fu

ryl), 123.13 (C-5), 143.32 (C-4), 143.45 (C-6), 144.58 (C-2 in furyl), 146.35 (C-5 in furyl) 146.66 (C-5 in furyl), 150.152 (C-2 in furyl), 155.38 (C-8), 158.55 (C-2).

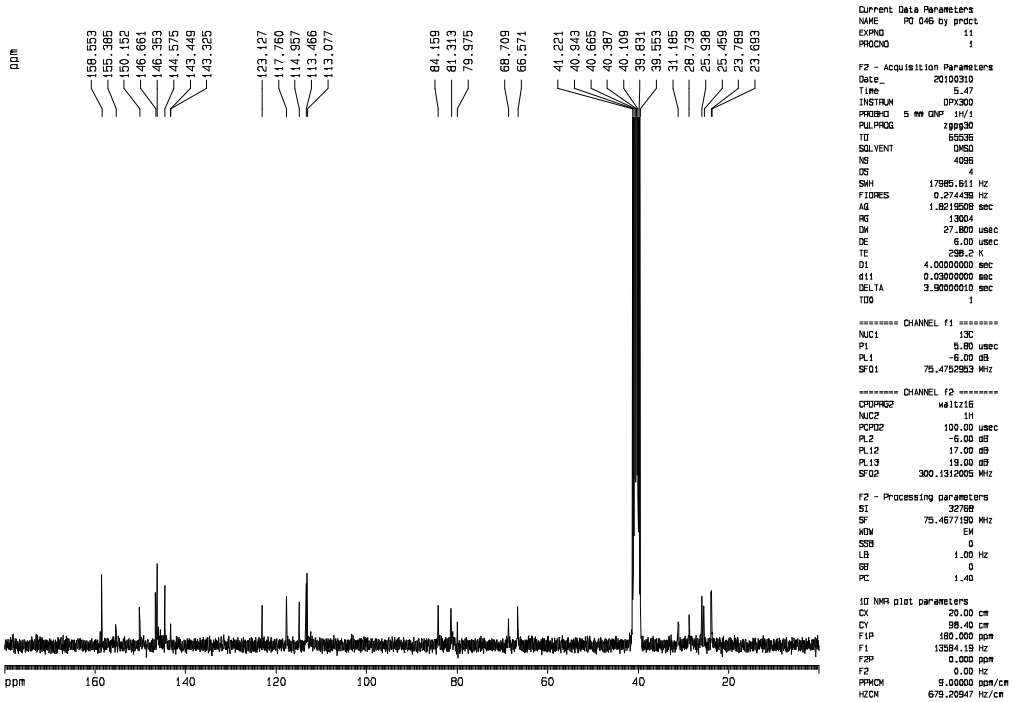
MS (ESI) m/z (rel. %)**:** 436 (50, M^+), 352 (100)

HR-MS: Found 435.190079, caculated value for $C_{23}H_{25}N_5O_4$ 435.1907.

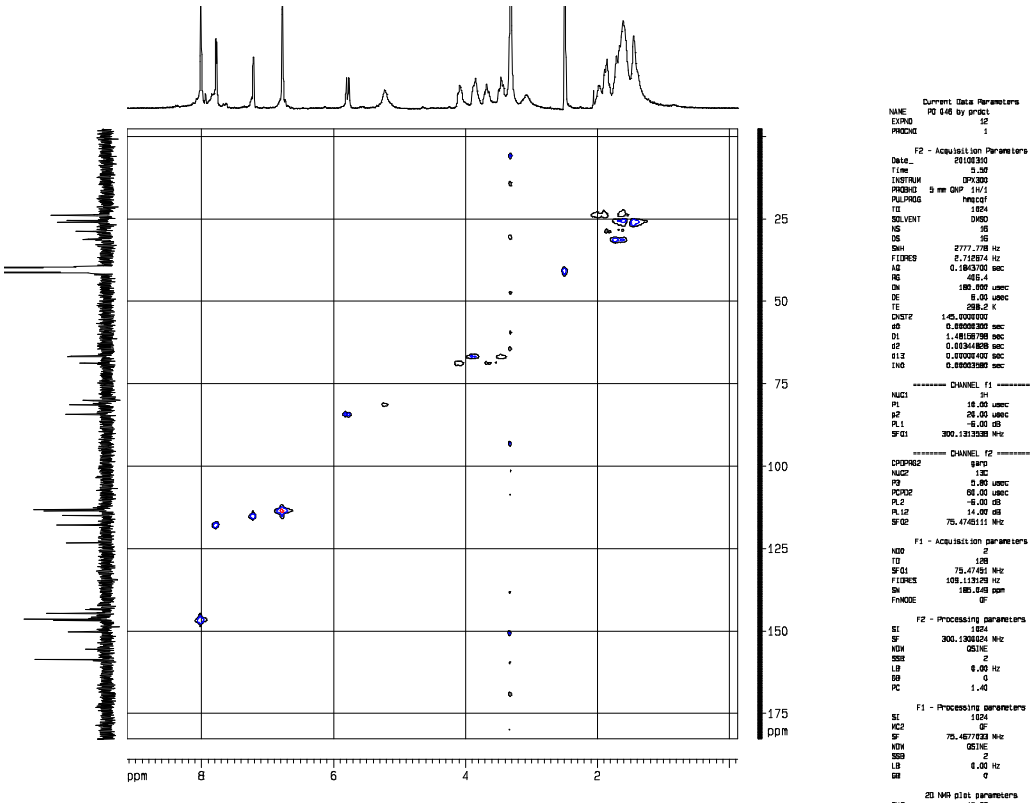


Spectrum 21. ^{1}H NMR of 6,8-di(furan-2-yl)-*N*,9-bis(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-2-amine **(35b).**

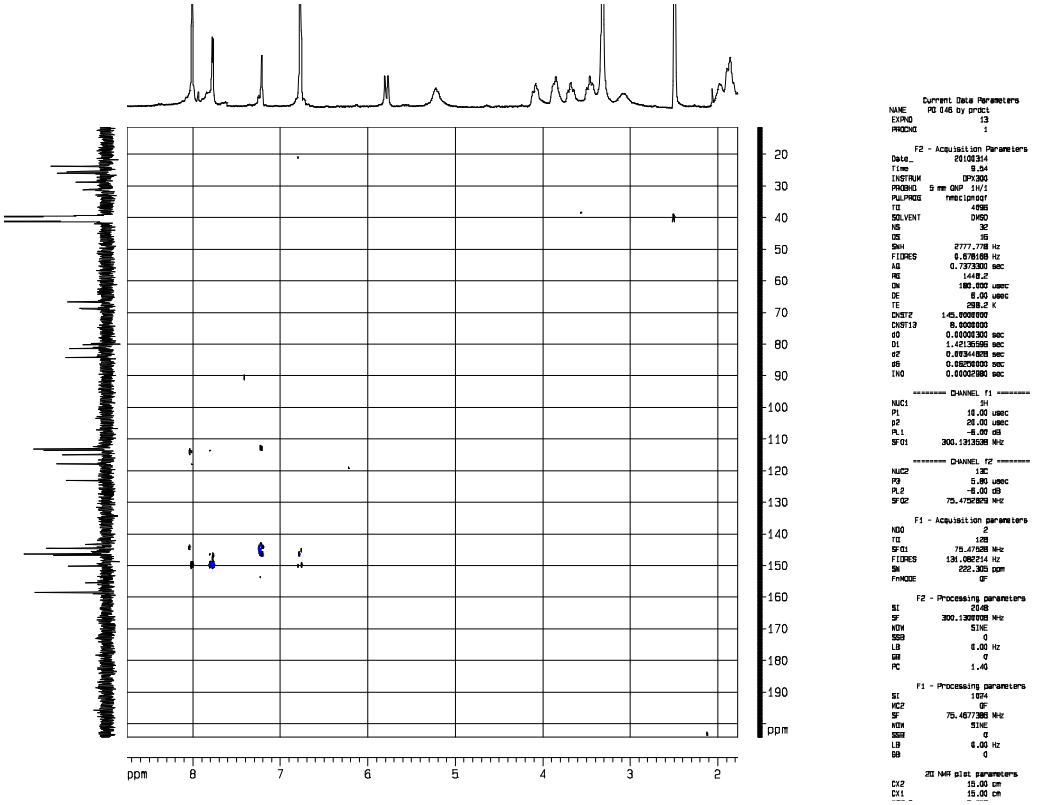
.



Spectrum 22. ^{13}C NMR of 6,8-di(furan-2-yl)-*N*,9-bis(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-2-amine **(35b).**

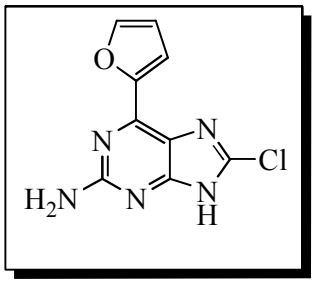


Spectrum 23. HMQC of 6,8-di(furan-2-yl)-*N*,9-bis(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-2-amine **(35b).**



Spectrum 24. HMBC of 6,8-di(furan-2-yl)-*N*,9-bis(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-2-amine **(35b).**

4.8 Synthesis of 8-Chloro-6-(furan-2-yl)-9*H*-purin-2-amine (38).



38

A mixture of 8-chloro-6-(furan-2-yl)-*N*,9-bis(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-2-amine **(35a)** (320 mg, 0.8 mmol), 96% EtOH (14 mL) and 9.6 M HCl (0.84 mL) was stirred at ambient temperature until TLC shows complete conversion and neutralized by addition of

solid $KHCO_3$. The resulting mixture was evaporated in vacuo and the product was isolated by flash chromatography on silica gel using Chloroform/MeOH (50:1).

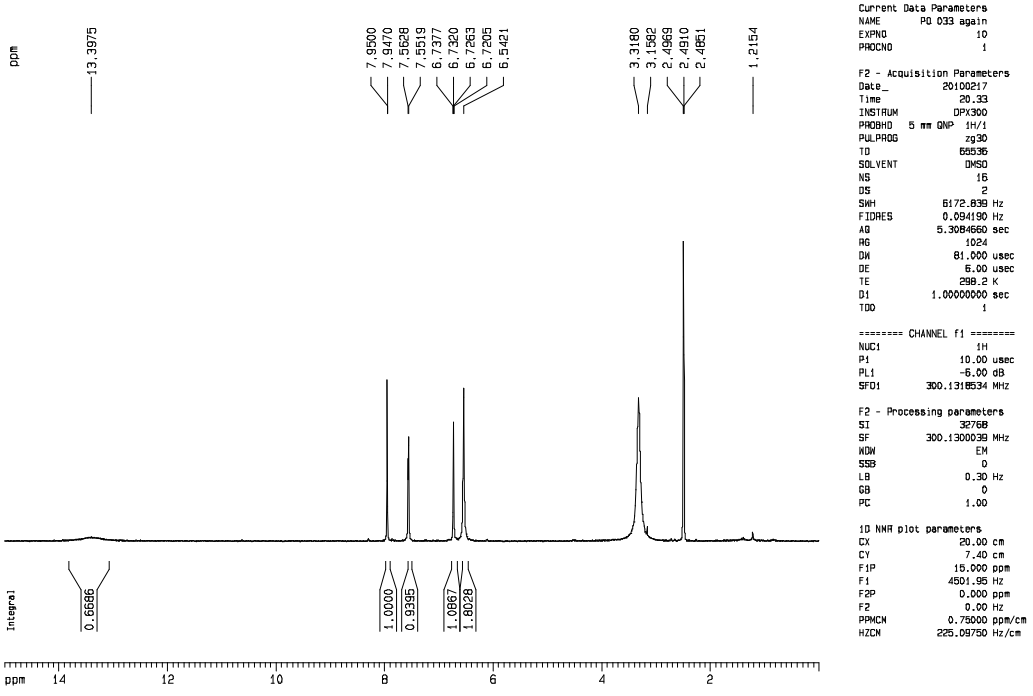
Yield 165 mg, (88 %), Yellowish powder. M.p 167 – 169 ^oC,

^{1}H NMR (DMSO- d_6, 300 MHz): δ 6.54 (s, 2H, NH_2), 6.73 (dd, J = 3.3, 1.7 Hz, 1H, H-4 in furyl), 7.56 (d, J = 3.27 Hz, 1H, H-3 in furyl), 7.95 (d, J = 0.9 Hz, H-5 in furyl), 13.40 (br, 1H, NH).

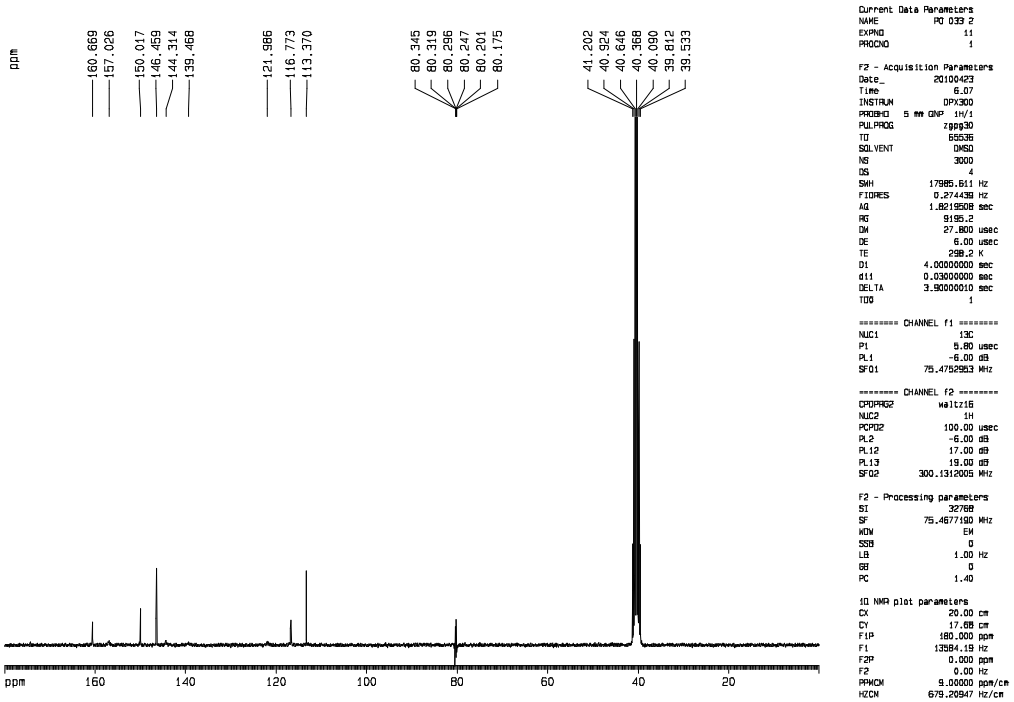
^{13}C NMR (DMSO- d_6, 75 MHz): δ 113.37 (C-4 in furyl), 116.77 (C-3 in furyl), 121.99 (C-5), 139.47 (C-4), 144.31 (C-6), 146.46 (C-5 in furyl), 150.02 (C-2 in furyl), 157.03 (C-8) 160.67 (C-2),

MS (EI) m/z (rel. %)**:** 237/235 (32/100, M^+)

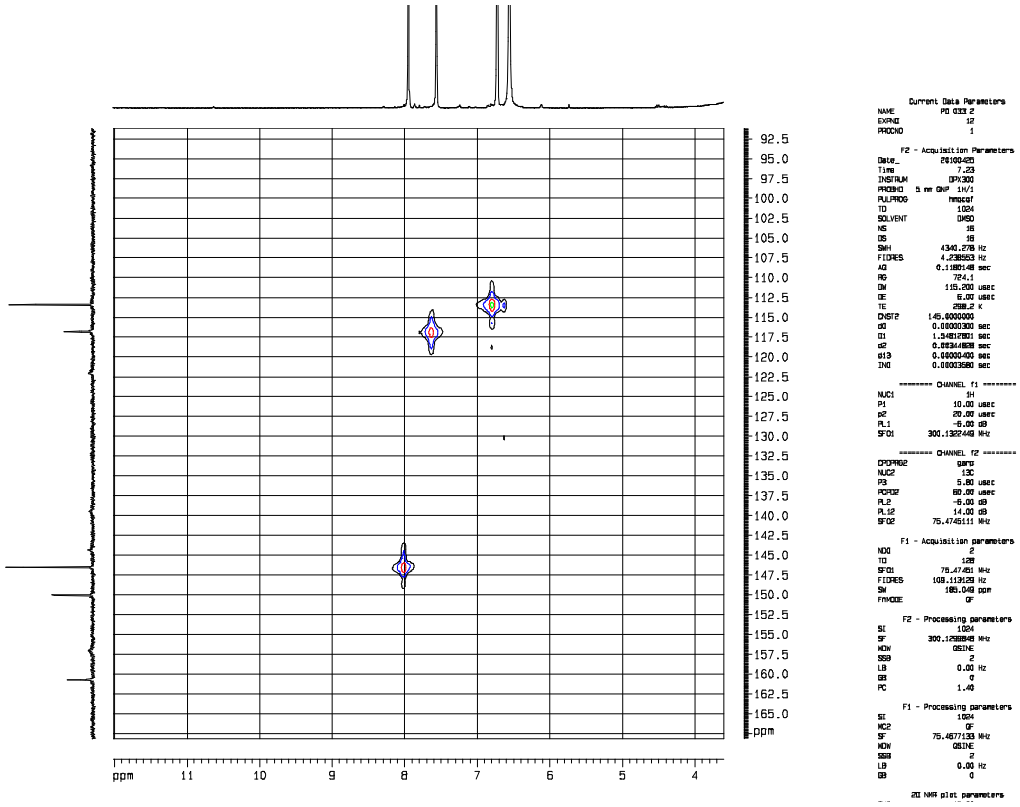
HR-MS: Found 235.025732, caculated value for $C_9H_6ClN_5O$ 235.0261.



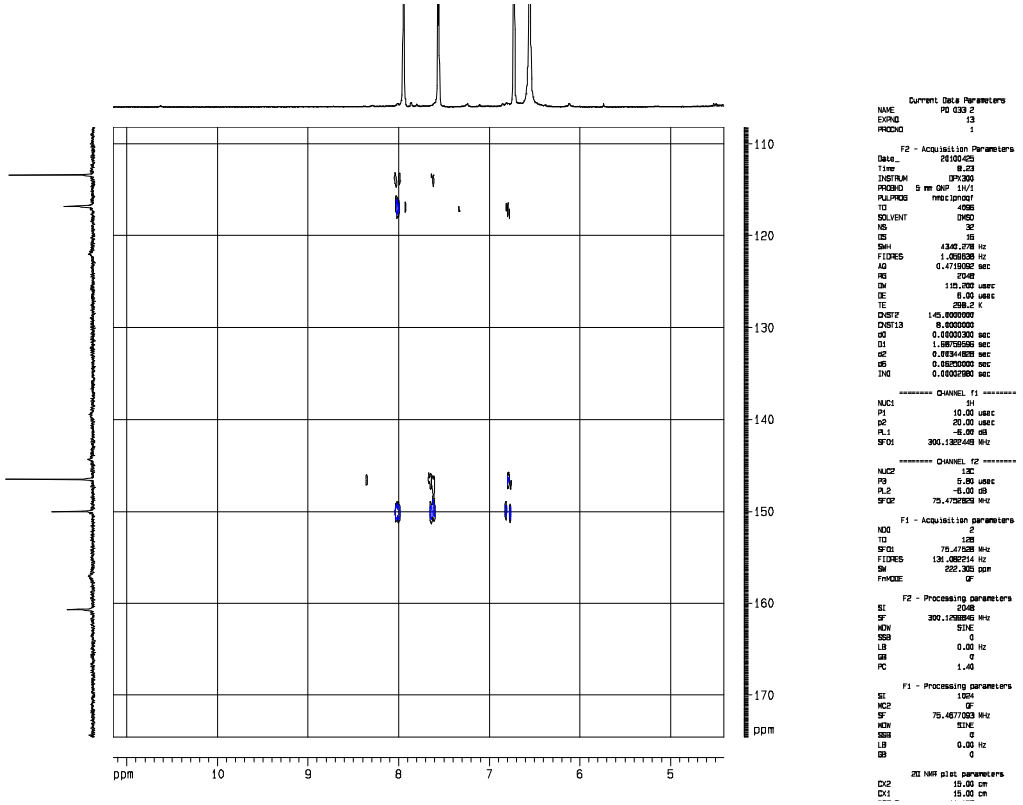
Spectrum 24. ^{1}H NMR of 8-Chloro-6-(furan-2-yl)-9*H*-purin-2-amine **(38).**



Spectrum 26. ^{13}C NMR of 8-Chloro-6-(furan-2-yl)-9*H*-purin-2-amine **(38).**

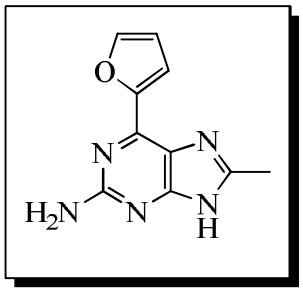


Spectrum 27. HMQC of 8-Chloro-6-(furan-2-yl)-9*H*-purin-2-amine **(38).**



Spectrum 28. HMBC of 8-Chloro-6-(furan-2-yl)-9*H*-purin-2-amine **(38).**

4.9 Synthesis of 6-(Furan-2-yl)-8-methyl-9*H*-purin-2-amine (40).



40

A mixture of 6-(furan-2-yl)-8-methyl-*N*,9-bis(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-2-amine **(37)** (150 mg, 0.4 mmol), 96% EtOH (7 mL) and 9.6 M HCl (0.4 mL) was stirred at ambient temperature until TLC shows complete conversion and neutralized by addition of solid

$KHCO_3$. The resulting mixture was evaporated *in vacuo* and the product was isolated by flash chromatography on silica gel using Chloroform/MeOH (50:1).

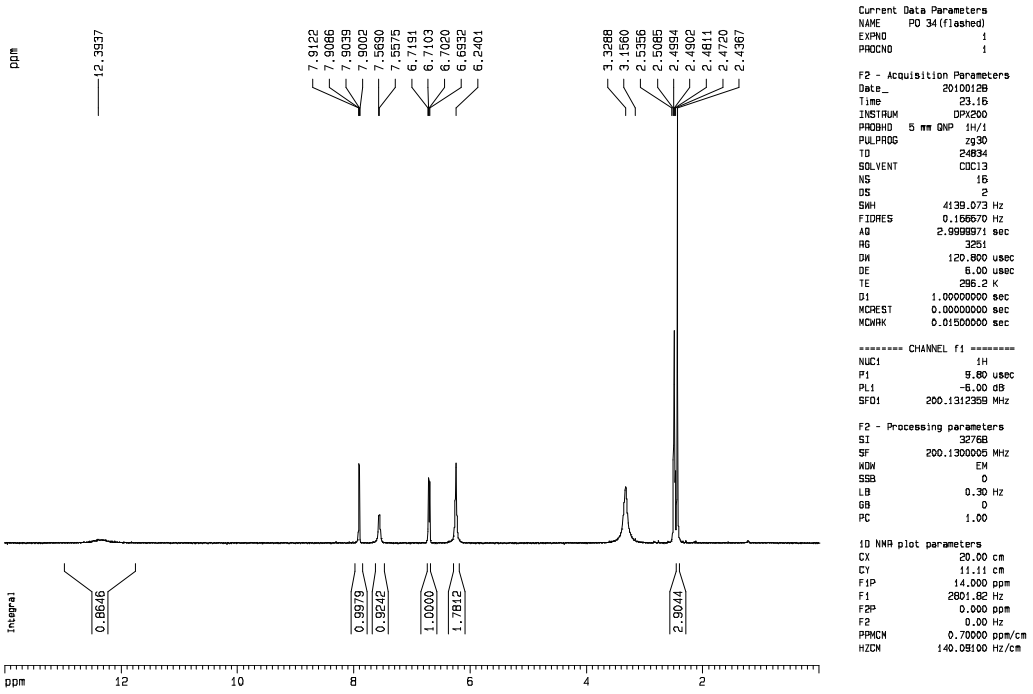
Yield 82 mg, (95 %), Yellowish powder. M.p 151 – 154 ^oC,

^{1}H NMR (DMSO- d_6, 200 MHz): δ 2.45 (s, 3H, CH_3), 6.24 (br s, 2H, NH_2), 6.71 (dd, J = 3.4, 1.7Hz, 1H, H-4 in furyl), 7.56 (d, J = 2.3, 1H, H-3 in furyl), 7.91 (dd, J = 1.66, 0.94 Hz, 1H, H-5 in furyl), 12.39 (br, 1H, NH)

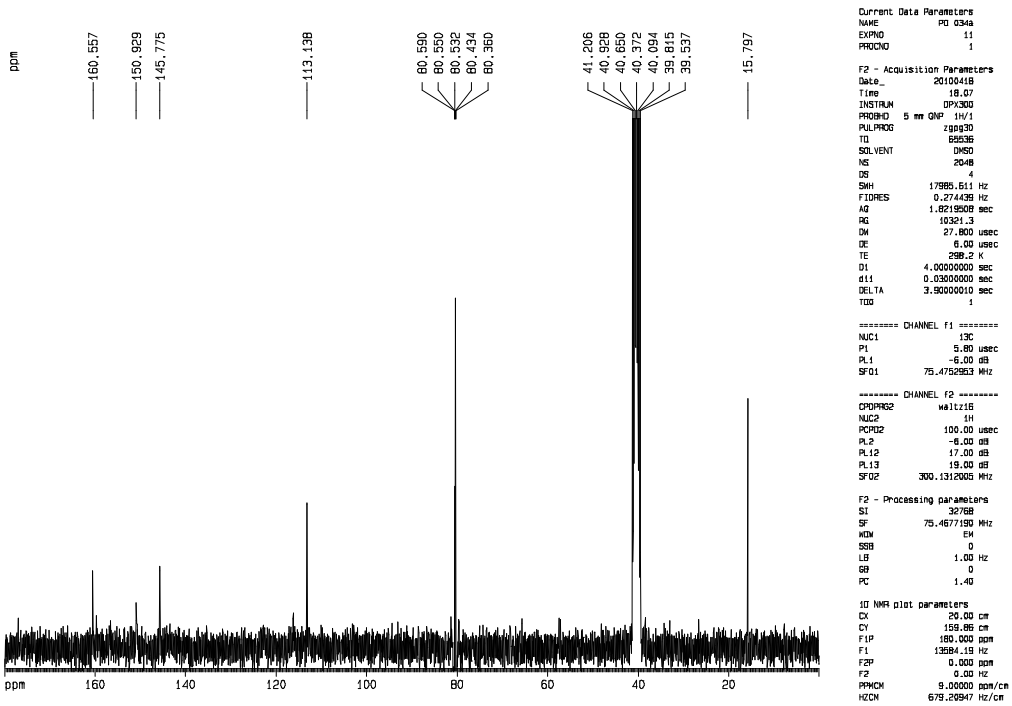
^{13}C NMR (DMSO- d_6, 75 MHz): δ 15.80 (CH_3) 113.14 (C-4 in furyl), 116.60 (C-3 in furyl), 145.77 (C-5 in furyl), 150.93 (C-2 in furyl), 160.56 (C-2),

MS (EI) m/z (rel. %)**:** 215 (100, M^+)

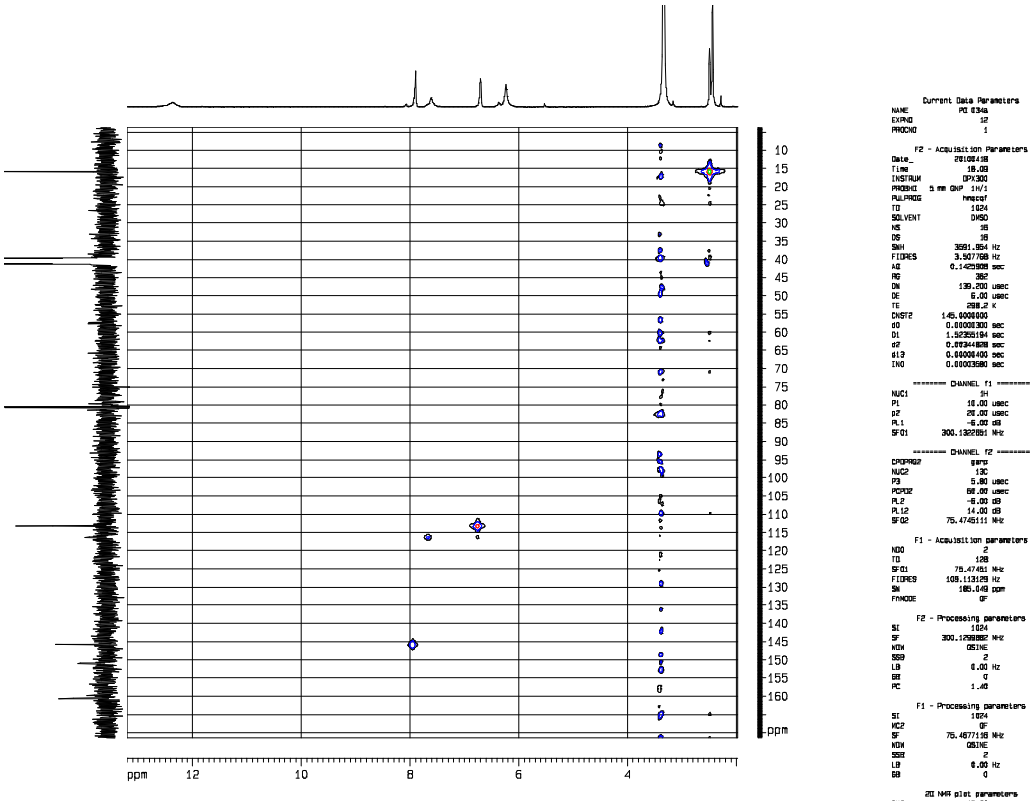
HR-MS: Found 215.080198, caculated value for $C_{10}H_9N_5O$ 215.0807.



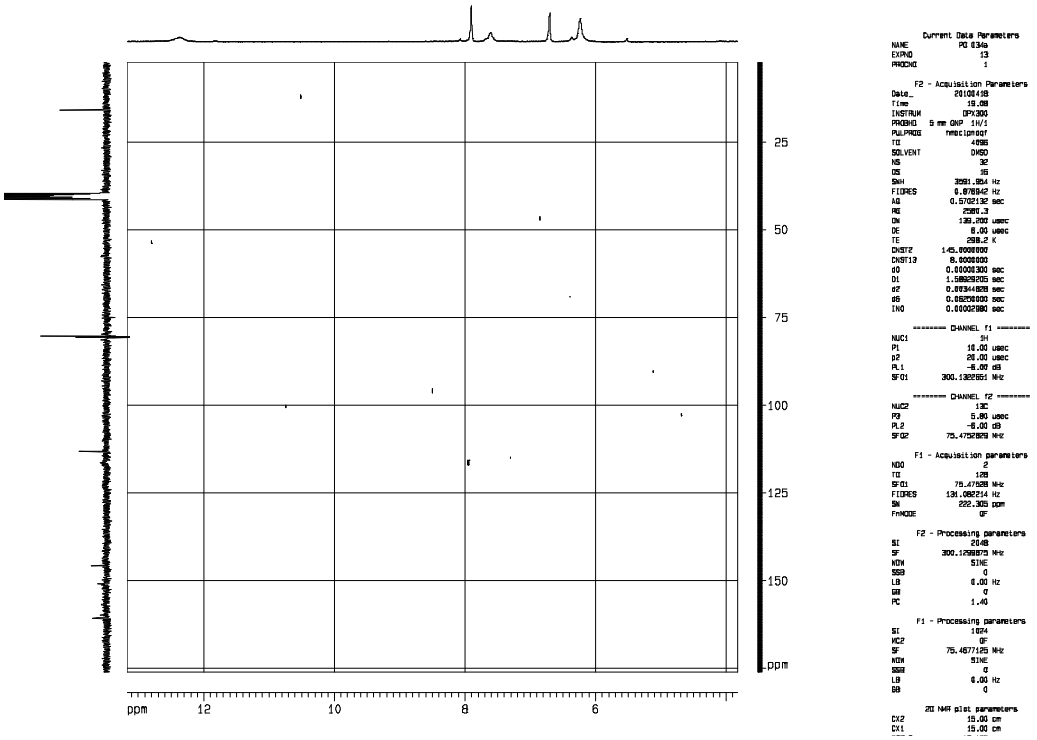
Spectrum 29. ^{1}H NMR of 6-(Furan-2-yl)-8-methyl-9*H*-purin-2-amine **(40).**



Spectrum 30. ^{13}C of 6-(Furan-2-yl)-8-methyl-9*H*-purin-2-amine (**40**).

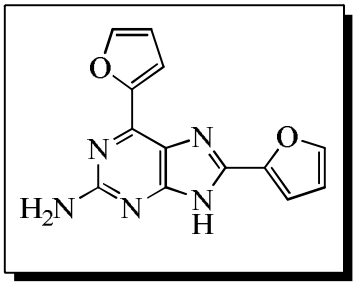


Spectrum 31. HMQC of 6-(Furan-2-yl)-8-methyl-9*H*-purin-2-amine (**40**)



Spectrum 32. HMBC of 6-(Furan-2-yl)-8-methyl-9*H*-purin-2-amine **(40).**

4.10. Synthesis of 6,8-di(furan-2-yl)-9*H*-purin-2-amine (39).



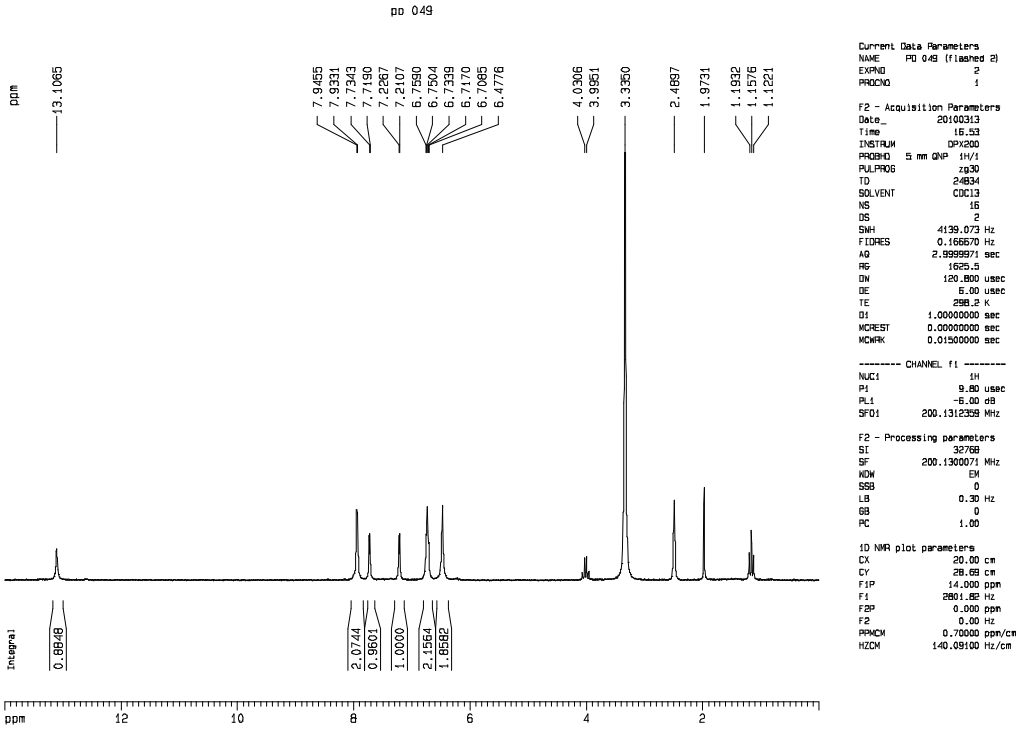
39

A mixture of 6,8-di(furan-2-yl)-*N*,9-bis(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-2-amine **(35b)** (220 mg, 0.51 mmol), 96% EtOH (10 mL) and 9.6 M HCl (0.55 mL) was stirred at ambient temperature until TLC shows complete conversion and neutralized by addition of solid $KHCO_3$. The resulting mixture was evaporated in vacuo and the product was isolated by flash chromatography on silica gel using Chloroform/MeOH (50:1).

Yield 98 mg, (71 %), Yellowish powder. M.p 148 – 151 ^{o}C,

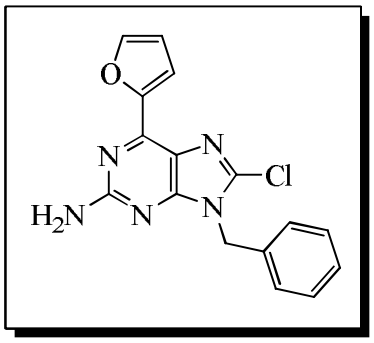
HR-MS: Found 267.080356, caculated value for $C_{13}H_9N_5O_2$ 267.0756.

^{1}H NMR (DMSO- d_6, 200 MHz): δ 6.48 (br s, 2H, NH_2), 6.70 - 6.76 (m, 2H, H-4 in furyl), 7.22 (d, *J* = 3.2 Hz, H-3 in furyl), 7.73 (d, J = 3.1 Hz, H-3 in furyl), 7.93 – 7.95 (m, H-5 in fruyl), 13.1065 (br s, 1H, NH).

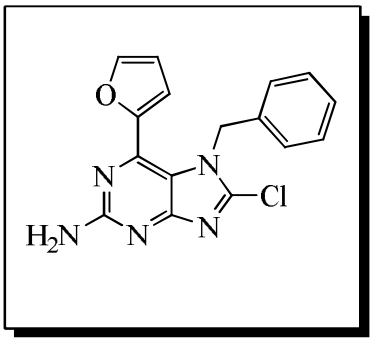


Spectrum 33. ^{1}H NMR of 6,8-di(furan-2-yl)-9*H*-purin-2-amine **(39).**

4.11. Synthesis of 9-Benzyl-8-chloro-6-(furan-2-yl)-9*H*-purin-2-amine (42a) and 7-benzyl-8-chloro-6-(furan-2-yl)-7*H*-purin-2-amine (42b)



42a



42b

Potassium carbonate (83 mg, 0.6 mmol) was added to a stirred solution of 8-chloro-6-(furan-2-yl)-9*H*-purin-2-amine **(38)** (50 mg, 0.2 mmol) in dry DMF (2mL) at ambient temperature under nitrogen. After 20 min benzyl chloride (0.036 mL, 0.3 mmol) was added, the resulting mixture was stirred for 15 hr, filtered and evaporated. The isomers were separated by flash chromatography on silica gel using EtOAc/hexane (3:1).

9-Benzyl-8-chloro-6-(furan-2-yl)-9*H*-purin-2-amine (42a)

Yield 30 mg, (45 %), Yellowish powder. M.p 161 -163 ^{o}C,

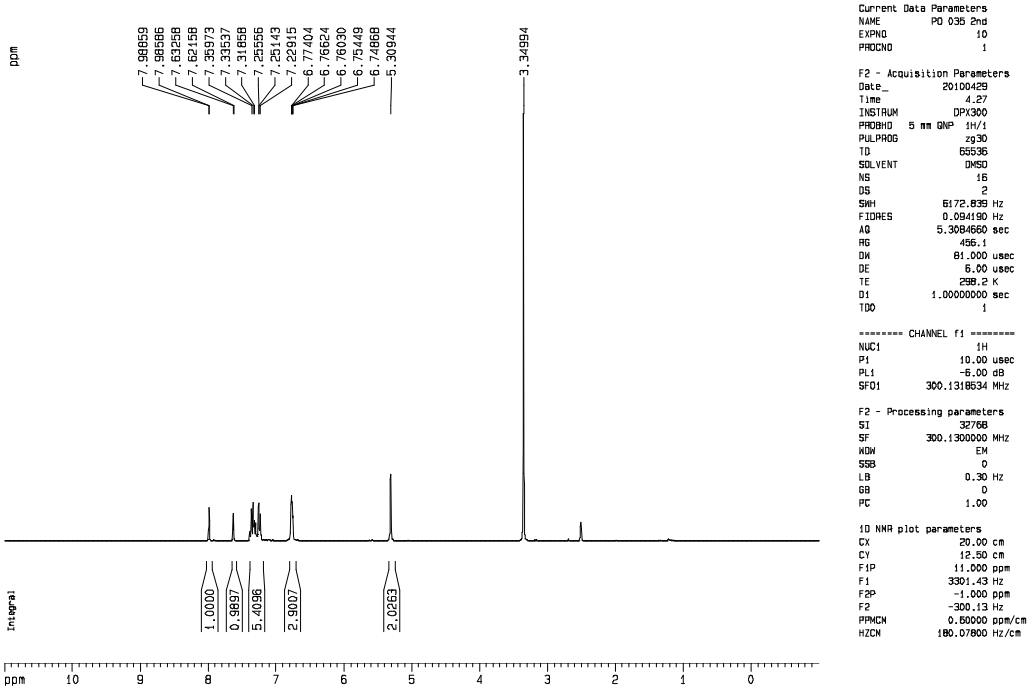
^{1}H NMR (DMSO- d_6, 300 MHz): δ 5.31 (s, 2H, CH_2), 6.75 - 6.77 (br, 3H, NH_2 and H-4 in furyl), 7.23 – 7.36 (m, 5H, Ph)**,** 7.63 (d, *J* = 3.3 Hz 1H, H-3 in furyl.), 7.99 (d, *J* = 0.81 Hz 1H, H-5 in furyl).

^{13}C NMR (DMSO- d_6, 75 MHz): δ 46.24 (CH_2), 113.44 (C-4 in furyl), 117.32 (C-3 in furyl), 121.12 (C-5), 127.74, 128.68, 129.68 (CH in Ph), 136.59 (C in Ph), 139.05 (C-4), 145.65 (C-6), 146.70 (C-5 in furyl), 149.87 (C-2 in furyl), 155.37 (C-8), 161.29 (C-2),

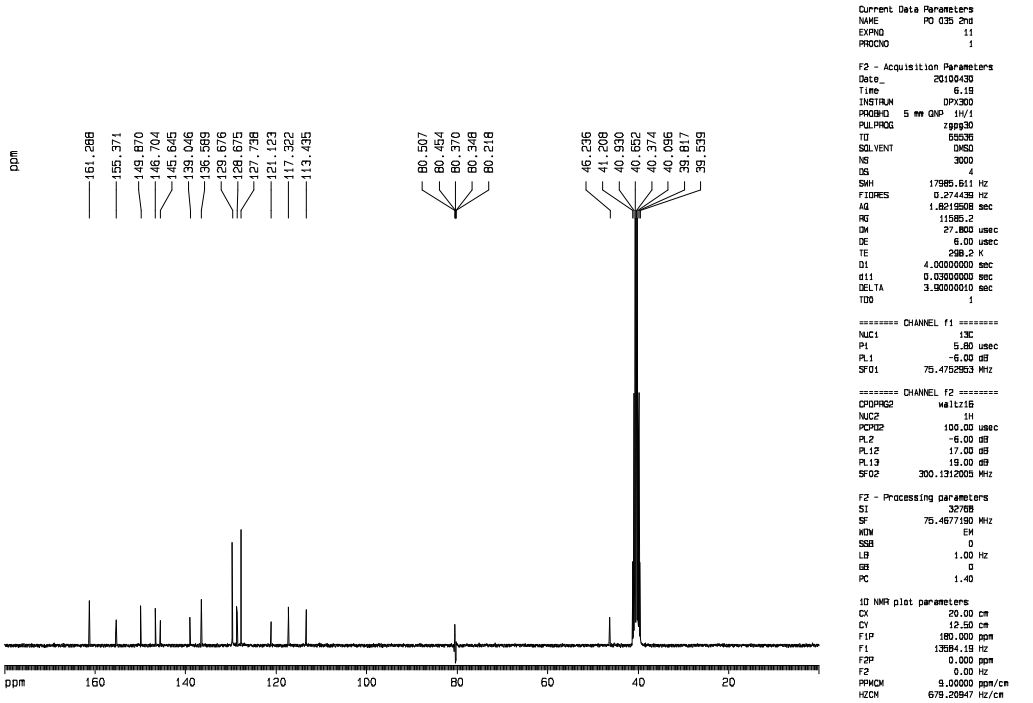
MS (EI) m/z (rel. %)**:** 327/325 (29/83, M^+), 290 (6), 234 (2), 91 (100),

HR-MS: Found 325.073645, caculated value for $C_{16}H_{12}ClN_5O$ 325.0730

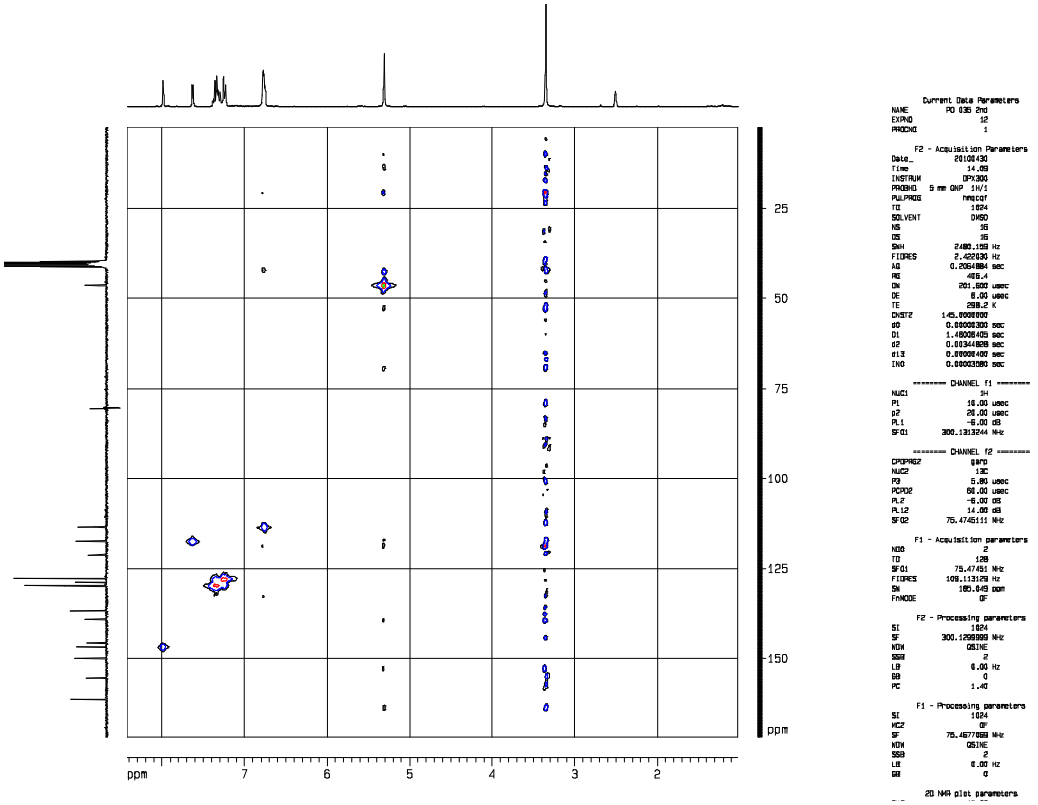
Anal: Found C, 59.46; H, 3.65; N, 21.90. $C_{16}H_{12}ClN_5O$ requires C, 58.99; H, 3.71; N, 21.50



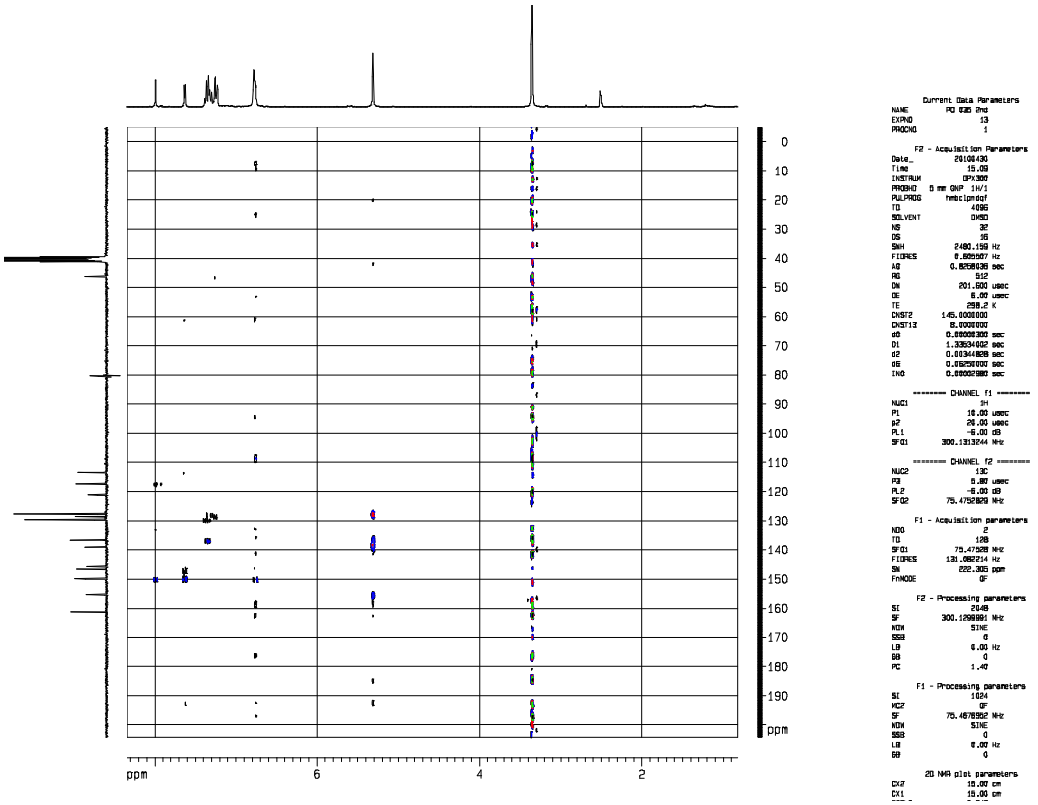
Spectrum 34. ^{1}H NMR of 9-Benzyl-8-chloro-6-(furan-2-yl)-9*H*-purin-2-amine **(42a).**



Spectrum 35. ^{13}C NMR of 9-Benzyl-8-chloro-6-(furan-2-yl)-9*H*-purin-2-amine **(42a).**



Spectrum 36. HMQC of 9-Benzyl-8-chloro-6-(furan-2-yl)-9*H*-purin-2-amine **(42a).**



Spectrum 37. HMBC of 9-Benzyl-8-chloro-6-(furan-2-yl)-9*H*-purin-2-amine **(42a).**

4.12. 7-benzyl-8-chloro-6-(furan-2-yl)-7*H*-purin-2-amine (42b)

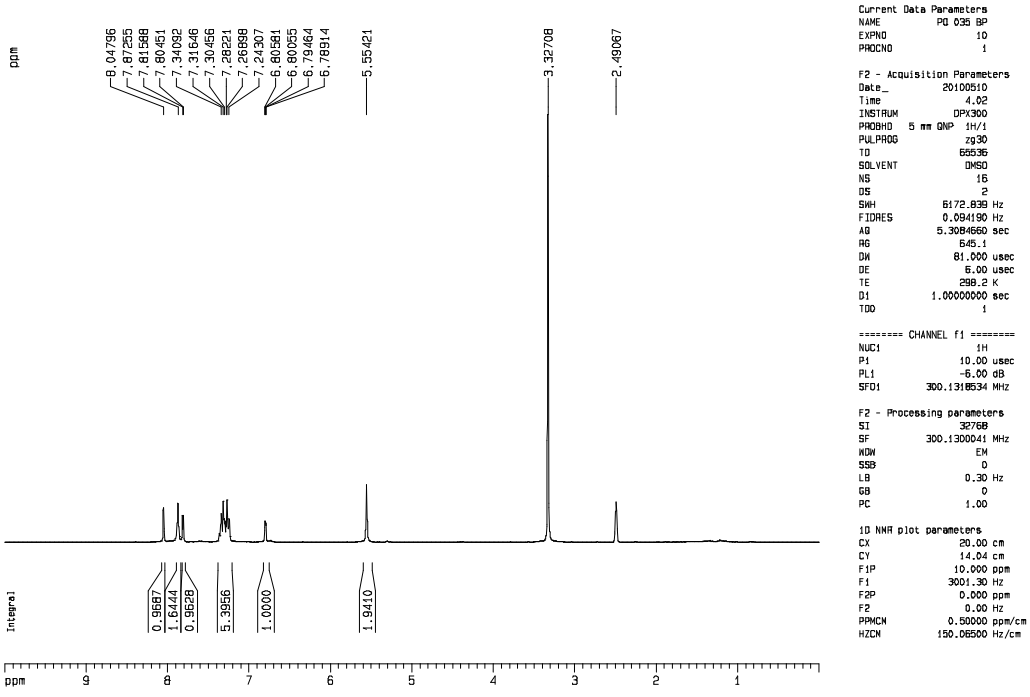
Yield (45 %), Yellowish powder. M.p 159 – 161 ^{o}C,

^{1}H NMR (DMSO- d_6, 300 MHz): δ 5.55 (s, 2H, CH_2), 6.80 (dd, J = 3.4, 1.8Hz, H-4 in furyl), 7.24 – 7.36 (m, 5H, Ph)**,** 7.81 (d, J = 3.4 Hz 1H, H-3 in furyl.), 7.87 (br s, 2H, NH_2), 8.17 (s, H-5 in furyl).

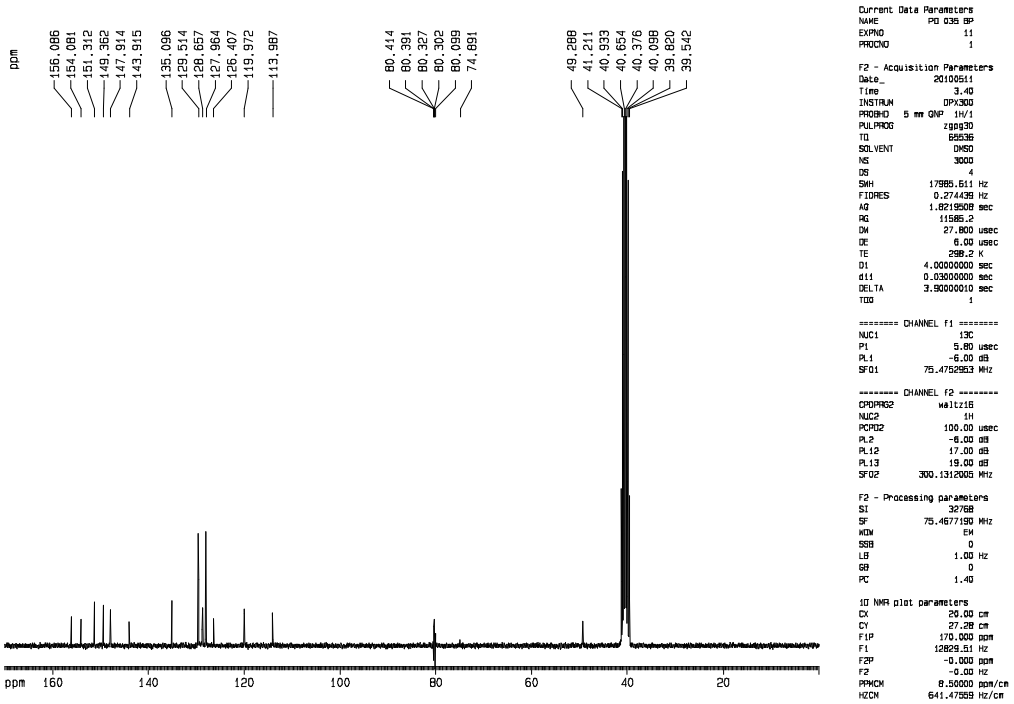
^{13}C NMR (DMSO- d_6, 75 MHz): δ 49.29 (CH_2), 113.99 (C-4 in furyl), 119.97 (C-3 in furyl), 126.41 (C-5), 127.96, 128.66, 129.51 (CH in Ph), 135.10 (C in Ph), 143.91 (C-4), 147-91 (C-5 in furyl), 149.36 (C-2 in furyl), 151.31 (C-5), 154.08 (C-2), 155.09 (C-8).

MS (EI) m/z (rel. %)**:** 325/327 (53/18, M^+), 91 (100),

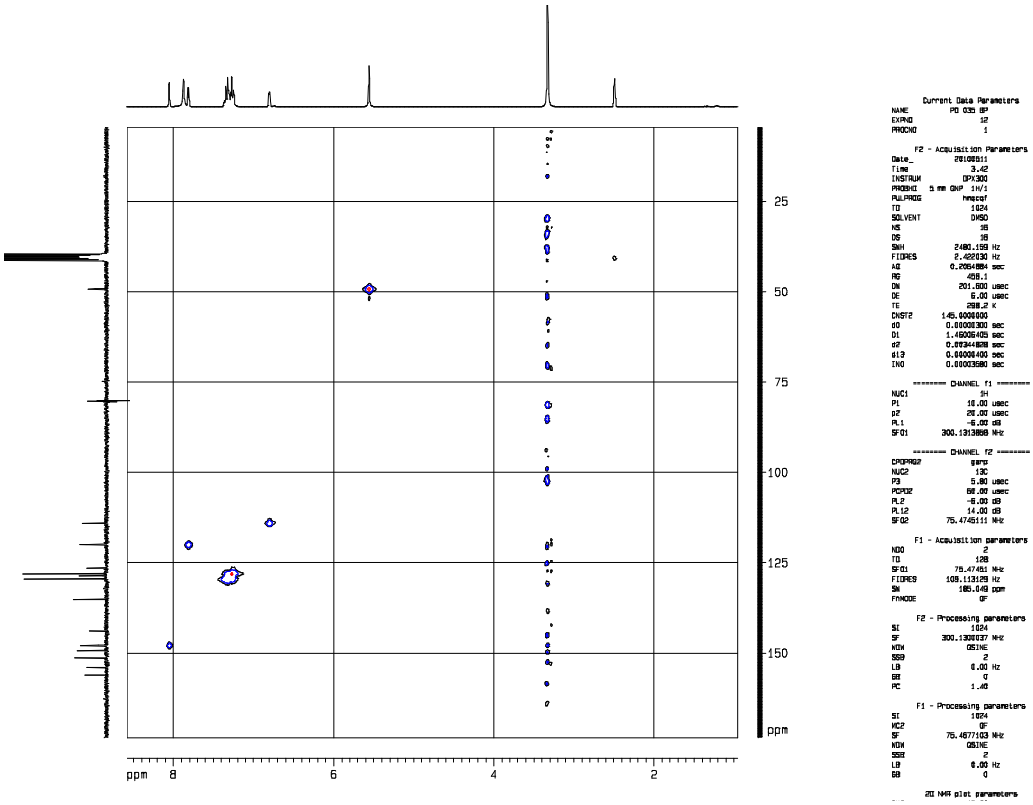
HR-MS: Found 325.073299, caculated value for $C_{16}H_{12}ClN_5O$ 325.0730



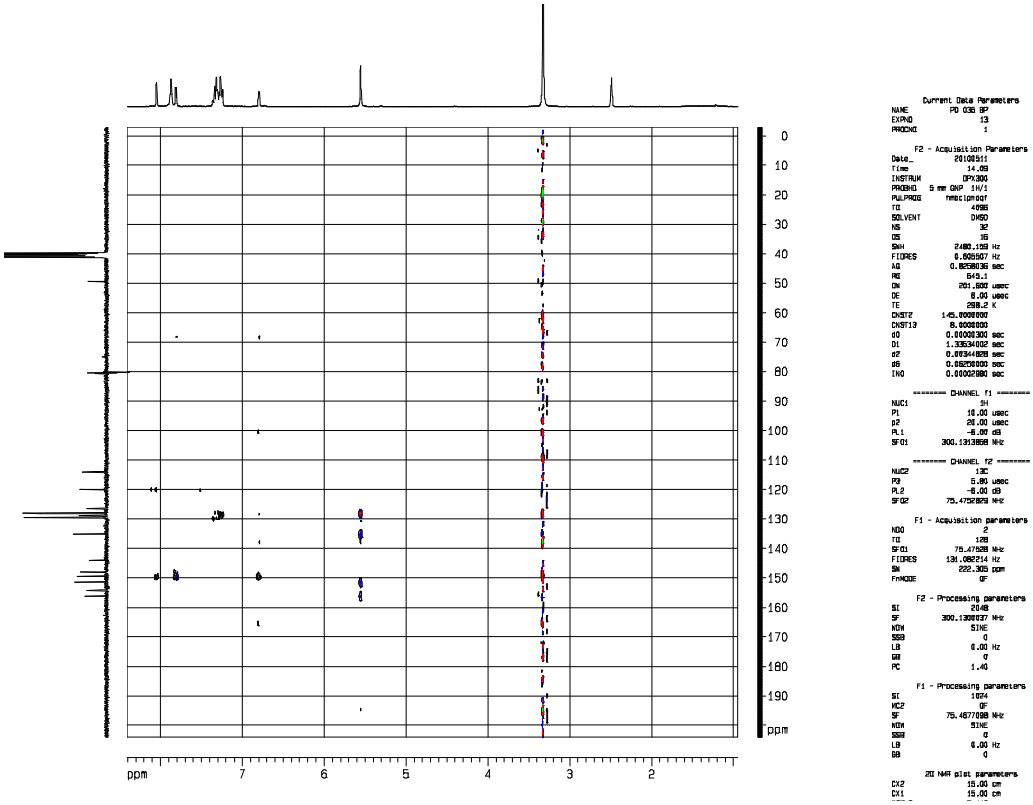
Spectrum 38. ^{1}H NMR of 7-benzyl-8-chloro-6-(furan-2-yl)-7*H*-purin-2-amine **(42b)**



Spectrum 39. ^{13}C NMR of 7-benzyl-8-chloro-6-(furan-2-yl)-7*H*-purin-2-amine **(42b)**

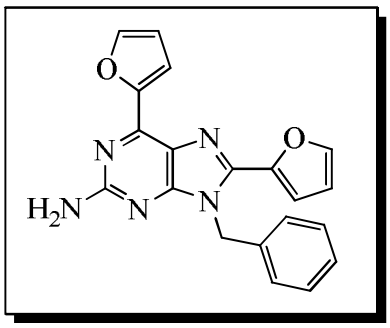


Spectrum 40. HMQC of 7-benzyl-8-chloro-6-(furan-2-yl)-7*H*-purin-2-amine **(42b)**

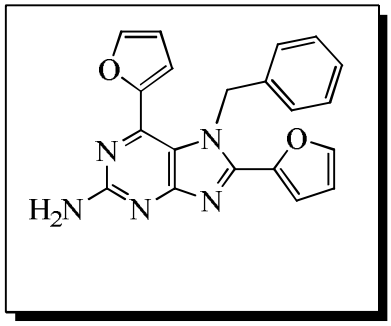


Spectrum 41. HMBC of 7-benzyl-8-chloro-6-(furan-2-yl)-7*H*-purin-2-amine **(42b)**

4.13. 9-benzyl-6,8-di(furan-2-yl)-9*H*-purin-2-amine (43a) and 7-benzyl-6,8-di(furan-2-yl)-7*H*-purin-2-amine (43b).



43a



43b

Potassium carbonate (80 mg, 0.57 mmol) was added to a stirred solution of 6,8-di(furan-2-yl)-9H-purin-2-amine **(39)** (50 mg, 0.19 mmol) in dry DMF (2mL) at ambient temperature under nitrogen. After 20 min benzyl chloride (0.035 mL, 0.29 mmol) was added, the resulting mixture was stirred for 15 hr, filtered and evaporated. The isomers were separated by flash chromatography on silica gel using EtOAc/hexane (3:1).

4.14. 9-benzyl-6,8-di(furan-2-yl)-9*H*-purin-2-amine (43a)

Yield 36mg (53 %), Yellowish powder. M.p 171 – 173 ^{o}C,

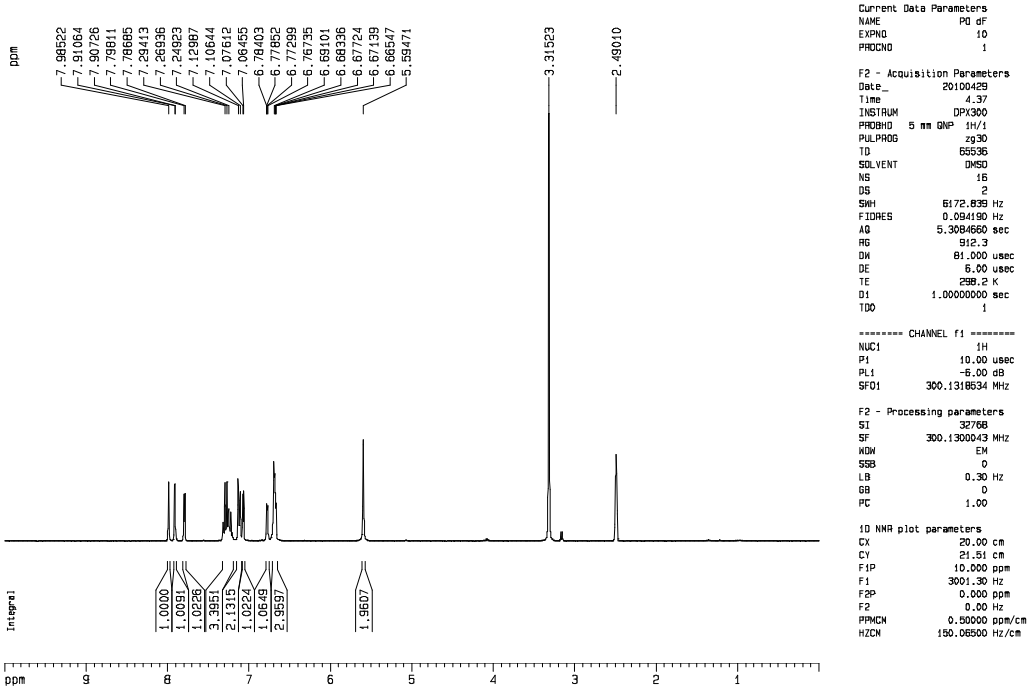
^{1}H NMR (DMSO- d_6, 300 MHz): δ 5.60 (s, 2H, CH_2), 6.67 - 6.69 (m, 3H, NH_2 and H-4 in furyl), 6.78 (dd, *J* = 3.3, 1.7Hz, 1H, H-4 in furyl), 7.07 (d, *J* = 3.5 Hz, 1H, H-3 in furyl.), 7.12 (d, *J* = 7.0 Hz, 2H, Ph), 7.25 – 7.29 (m, 3H, Ph)**,** 7.79 (d, *J* = 3.4 Hz, 1H, H-3 in furyl.), 7.91 (d, *J* = 1.0 Hz 1H, H-5 in furyl), 7.99 (s, 1H, H-5 in furyl).

^{13}C NMR (DMSO- d_6, 75 MHz): δ 46.52 (CH_2), 113.11 (C-4 in furyl), 113.41 (C-4 in furyl), 113.50 (C-3 in furyl), 117.52 (C-3 in furyl), 122.42 (C-5), 127.07, 128.27, 129.56 (CH in Ph), 137.72 (C in Ph), 142.70 (C-4), 145.04 (C-2 in furyl), 146.02 (C-5 in furyl), 146.08 (C-5 in furyl), 146.47 (C-6), 150.34 (C-2 in furyl), 156.20 (C-8), 161.36 (C-2).

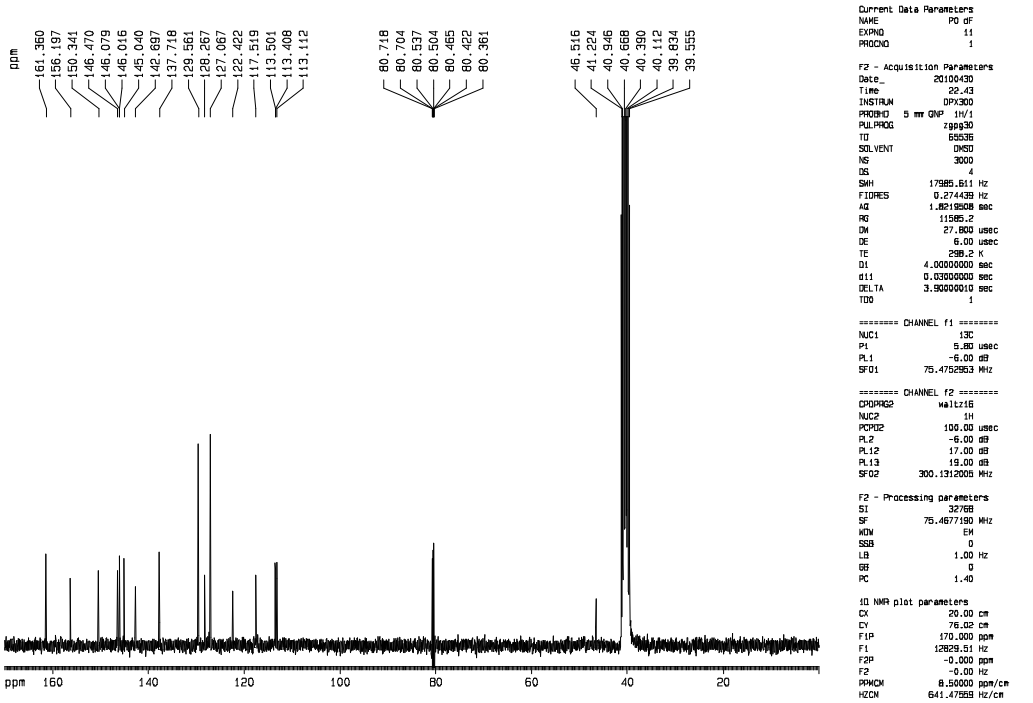
MS (EI) m/z (rel. %)**:** 357 (100, M^+), 266 (76), 91 (28).

HR-MS: Found 357.122012, caculated value $C_{20}H_{15}N_5O_2$ 357.1226

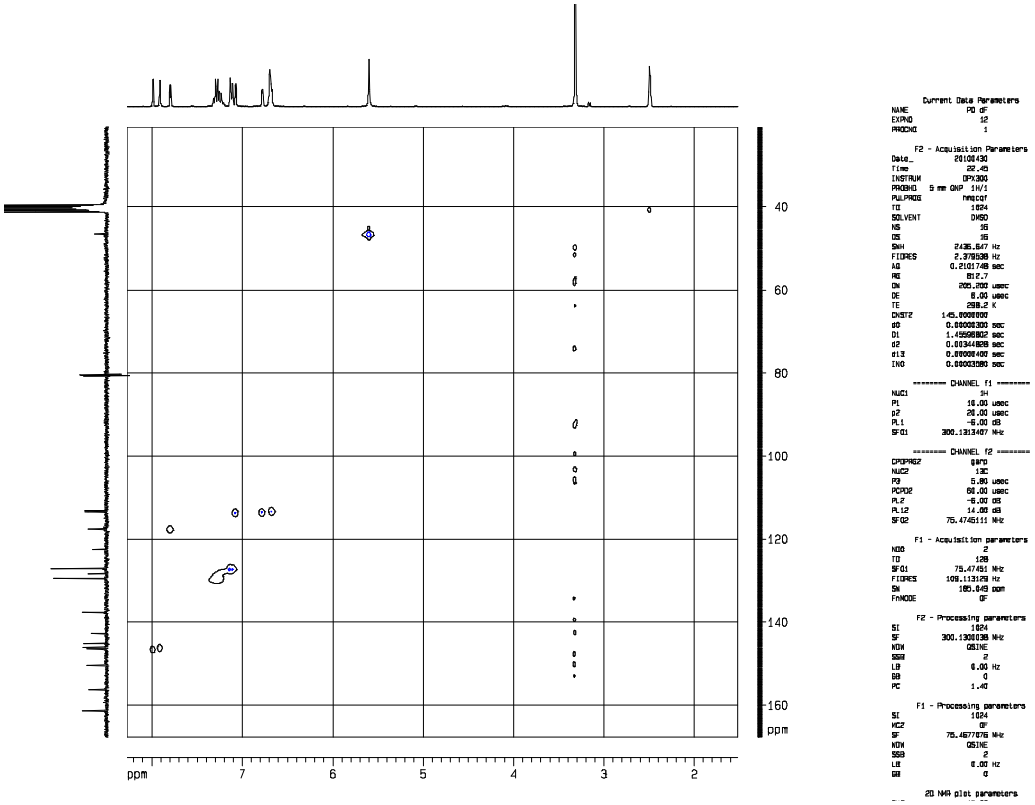
Anal: Found C, 67.34; H, 3.70; N, 19.83. $C_{20}H_{15}N_5O_2$ requires C, 67.22; H, 4.23; N, 19.60



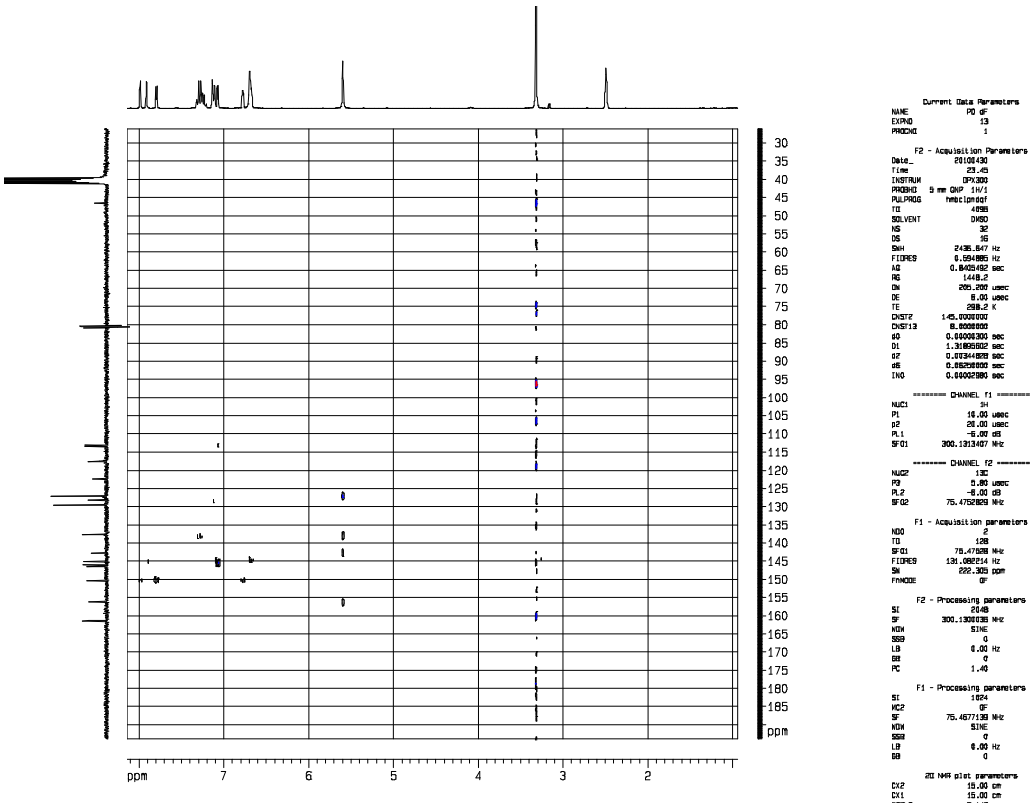
Spectrum 42. ^{1}H NMR of 9-benzyl-6,8-di(furan-2-yl)-9*H*-purin-2-amine **(43a).**



Spectrum 43. ^{13}C NMR of 9-benzyl-6,8-di(furan-2-yl)-9*H*-purin-2-amine **(43a).**



Spectrum 44. HMQC of 9-benzyl-6,8-di(furan-2-yl)-9*H*-purin-2-amine **(43a).**



Spectrum 45. HMBC of 9-benzyl-6,8-di(furan-2-yl)-9*H*-purin-2-amine **(43a)**

4.15. 7-benzyl-6,8-di(furan-2-yl)-7*H*-purin-2-amine (43b).

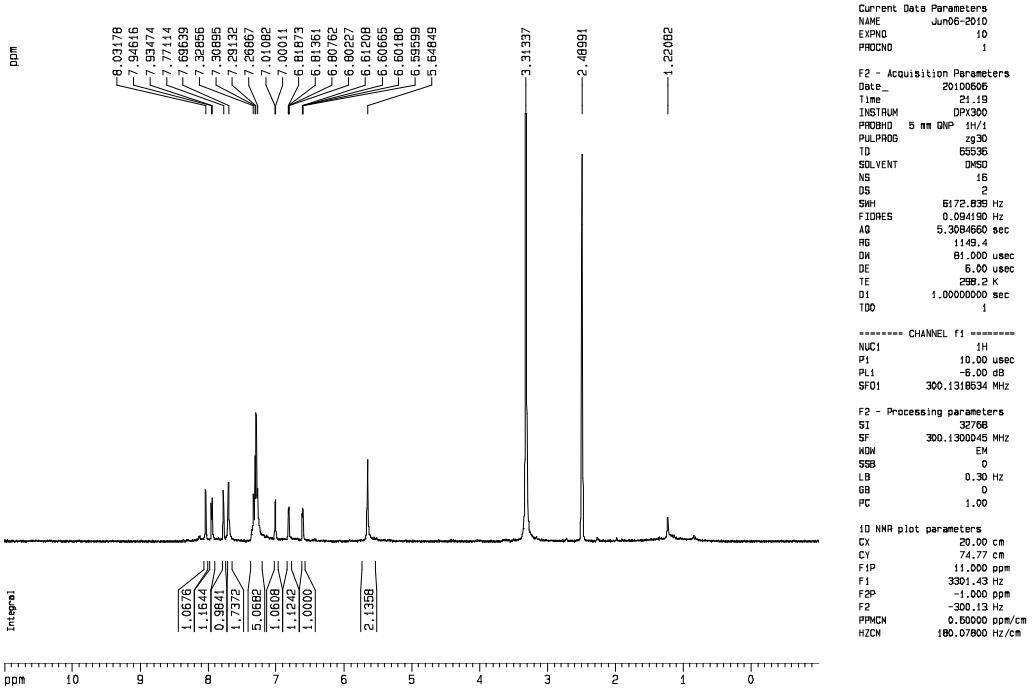
Yield (21 %), Yellowish powder. M.p 173 - 175 ^{o}C

^{1}H NMR (DMSO- d_6, 300 MHz): δ 5.65 (s, 2H, CH_2), 6.60 (dd, *J* = 3.1, 1.5Hz, 1H, H-4 in furyl), 6.81 (dd, *J* = 3.3, 1.8Hz, 1H, H-4 in furyl), 7.01 (d, *J* = 3.2 Hz, 1H, H-3 in furyl.), 7.27 – 7.35 (m, 5H, Ph)**,** 7.70 (br s, 2H, NH_2), 7.77 (s, 1H, H-5 in furyl), 7.94 (d, *J* = 3.4 Hz, 1H, H-3 in furyl.), 8.03 (s, 1H, H-5 in furyl).

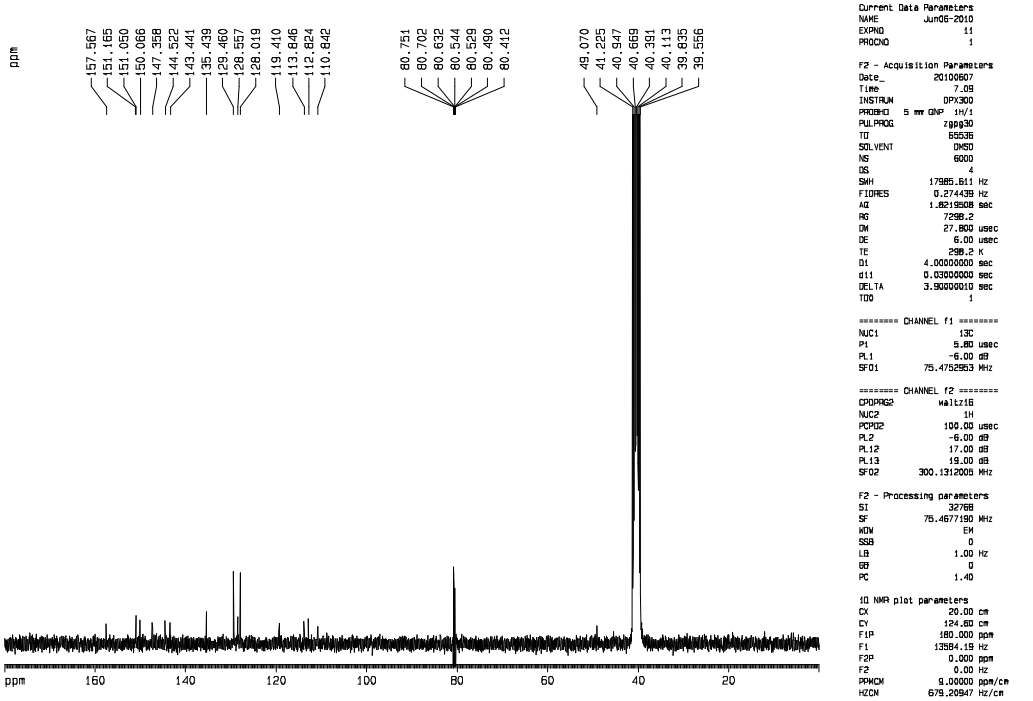
^{13}C NMR (DMSO- d_6, 75 MHz): δ 49.07 (CH_2), 110.84 (C-4 in furyl), 112.82 (C-4 in furyl), 113.85 (C-3 in furyl), 119.41 (C-3 in furyl), 128.02 (C-5), 128.56, 129.46, 135.44 (CH in Ph), 143.44 (C-4), 144.52 (C-2 in furyl), 147.36 (C-5 in furyl), 150.07 (C-5 in furyl), 151.05 (C-6), 151.16 (C-2 in furyl), 157.57 (C-8),

MS (EI) m/z (rel. %)**:** 357 (70, M^+), 266 (71), 91 (100).

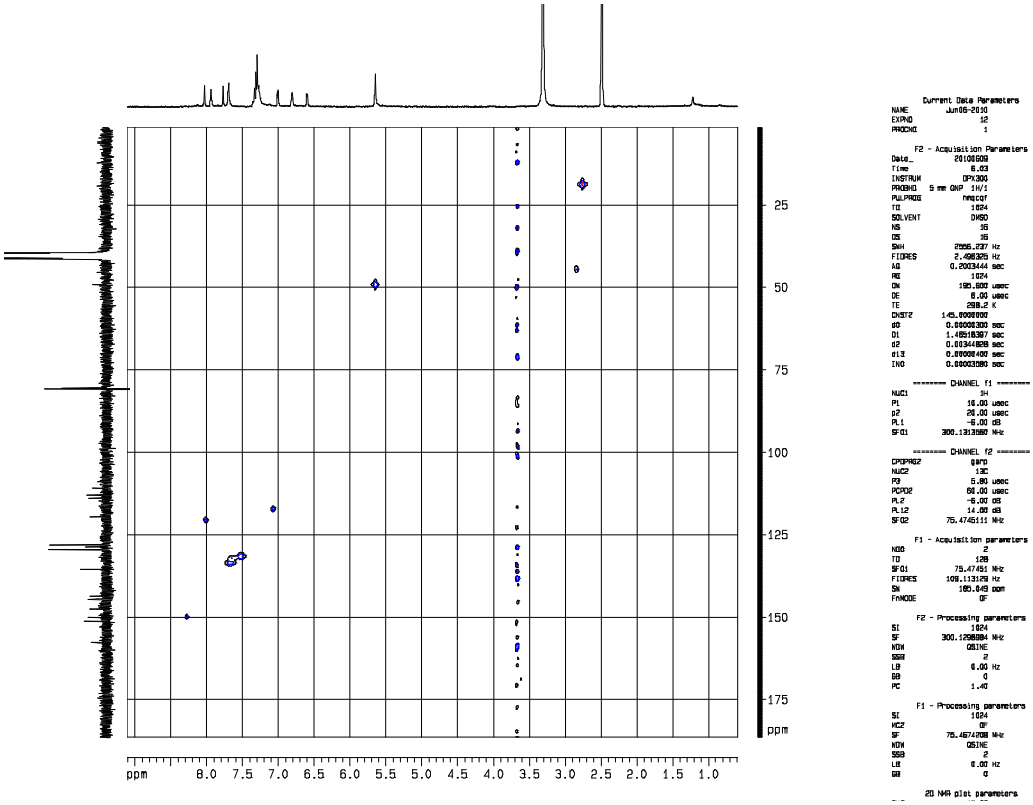
HR-MS: Found 357.122959, caculated value $C_{20}H_{15}N_5O_2$ 357.1226.



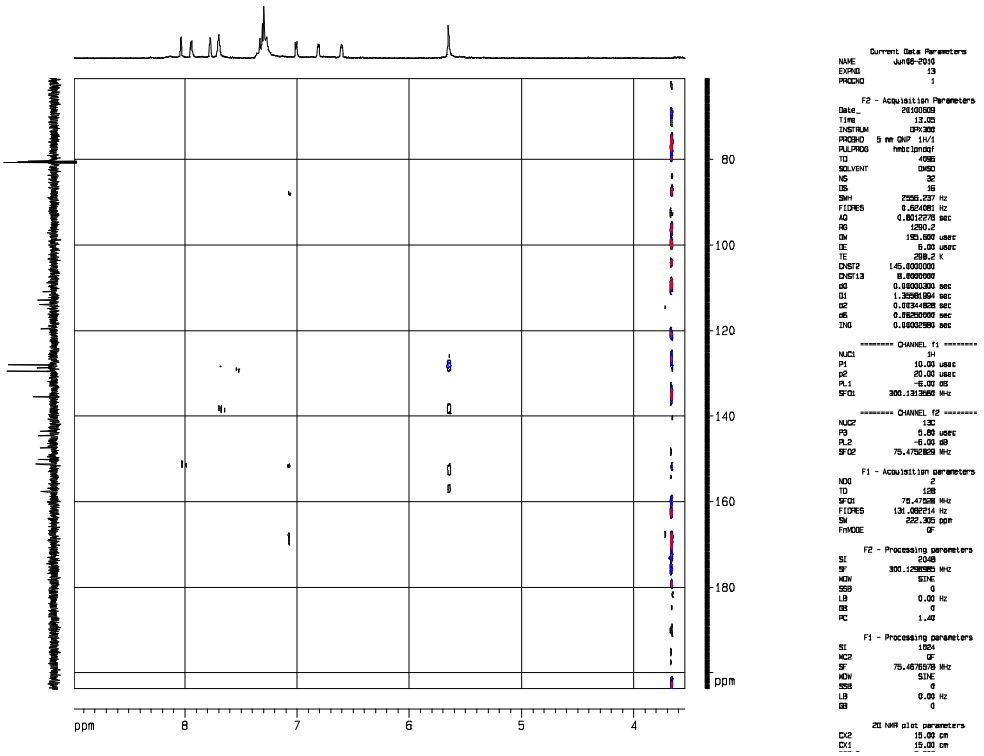
Spectrum 46. ^{1}H NMR of 7-benzyl-6,8-di(furan-2-yl)-7*H*-purin-2-amine **(43b).**



Spectrum 47. ^{13}C NMR of 7-benzyl-6,8-di(furan-2-yl)-7*H*-purin-2-amine **(43b).**

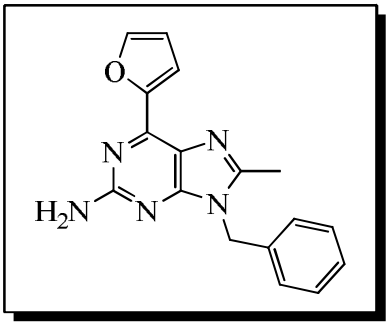


Spectrum 48. HMQC of 7-benzyl-6,8-di(furan-2-yl)-7*H*-purin-2-amine **(43b).**



Spectrum 49. HMBC of 7-benzyl-6,8-di(furan-2-yl)-7*H*-purin-2-amine **(43b).**

4.16 9-benzyl-6-(furan-2-yl)-8-methyl-9*H*-purin-2-amine (44a).



44a

Potassium carbonate (110 mg, 0.8 mmol) was added to a stirred solution of 6-(furan-2-yl)-8-methyl-9H-purin-2-amine **(40)** (55 mg, 0.26 mmol) in dry DMF (2mL) at ambient temperature under nitrogen. After 20 min benzyl chloride (0.06 mL, 0.52 mmol) was added, the resulting mixture was stirred for 15 hr, filtered and evaporated. TheN-9 isomer was isolated by flash chromatography on silica gel using EtOAc/hexane (2:1).

Yield: 53 mg, (65 %), Yellowish powder. M.p 151 – 155 ^{o}C

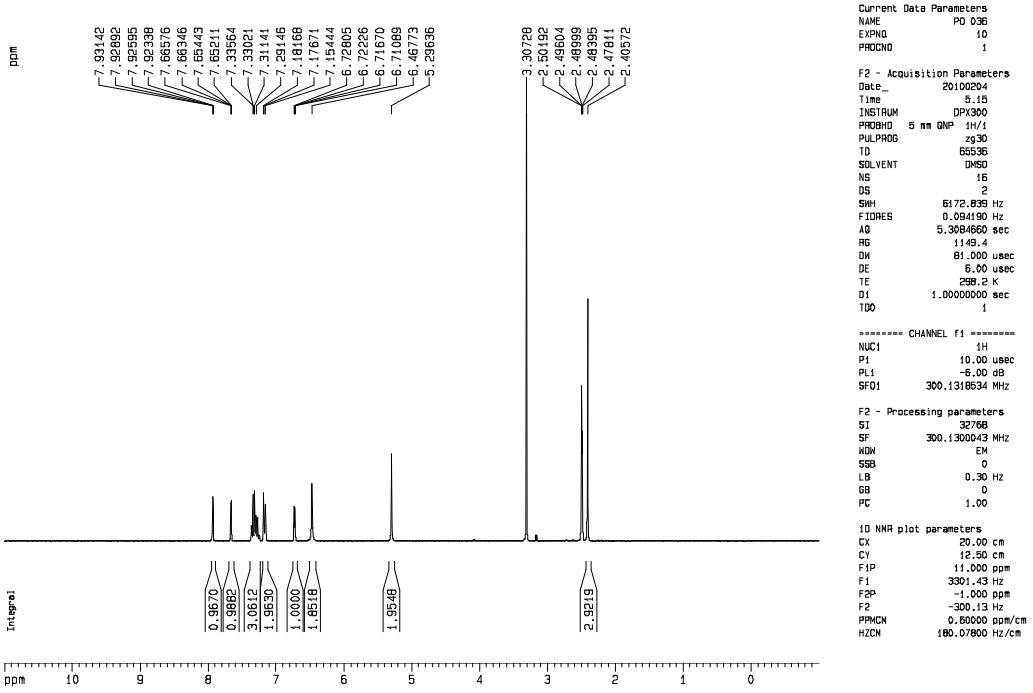
^{1}H NMR (DMSO- d_6, 300 MHz): δ 2.41 (s, 3H, CH_3), 5.30 (s, 2H, CH_2), 6.47 (s, 2H, NH_2), 6.72 (dd, *J* = 3.4, 1.7Hz, 1H, H-4 in furyl), 7.15 – 7.18 (m, 2H, Ph), 7.29 – 7.33 (m, 3H, Ph) 7.66 (dd, *J* = 3.4, 2.7Hz, 1H, H-3 in furyl), 7.91 (dd, *J* = 1.6, 0.9 Hz, 1H, H-5 in furyl).

^{13}C NMR (DMSO- d_6, 75 MHz): δ 14.85 (CH_3), 45.27 (CH_2), 113.20 (C-4 in furyl), 116.64 (C-3 in furyl), 121.86 (C-5), 127.54, 128.42, 129.66 (CH in Ph), 137.55 (C in Ph), 144.86 (C-6), 146.02 (C-5 in furyl), 150.63 (C-2 in furyl), 151.42 (C-8), 156.14 (C-4), 160.76 (C-2).

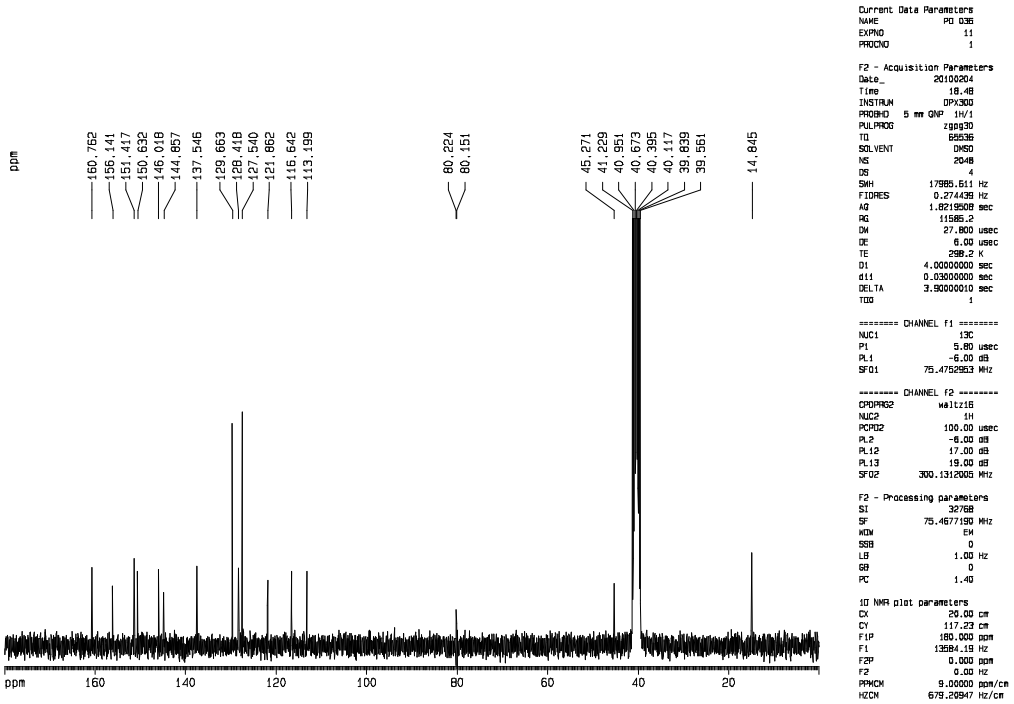
MS (EI) m/z (rel. %)**:** 305 (100, M^+),

HR-MS: Found 305.126800, caculated value for $C_{17}H_{15}N_5O$ 305.1277

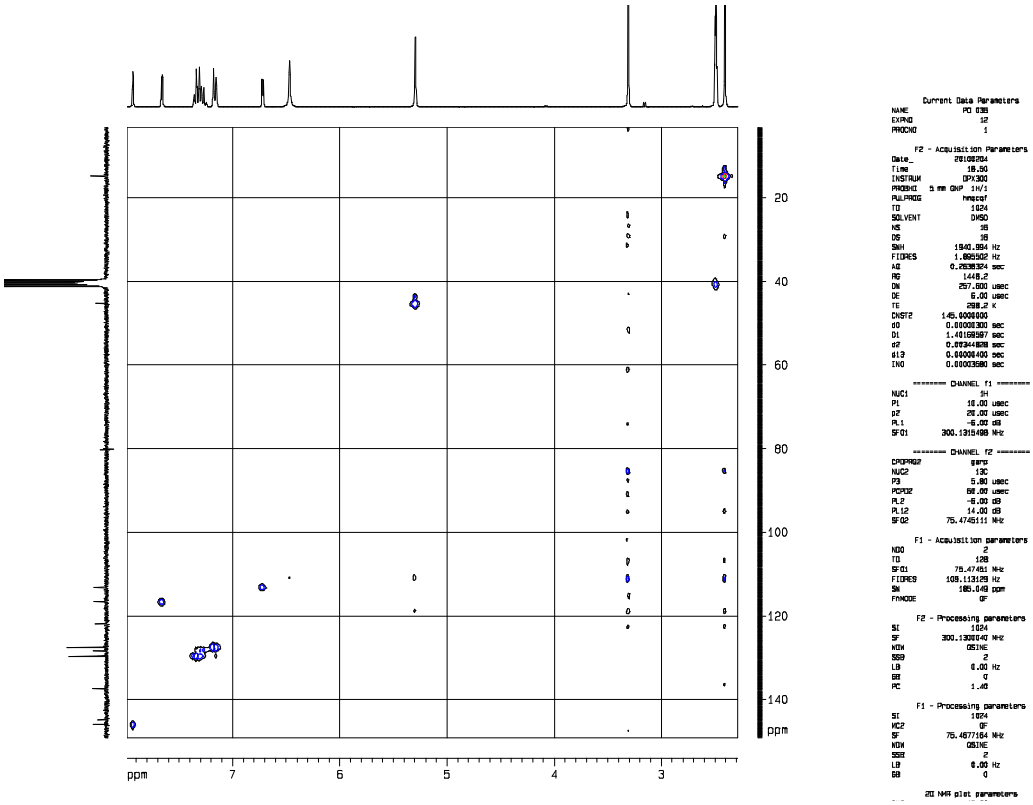
Anal: Found C, 66.26; H, 4.80; N, 23.13. $C_{16}H_{12}ClN_5O$ requires C, 66.87; H, 4.95; N, 22.94.



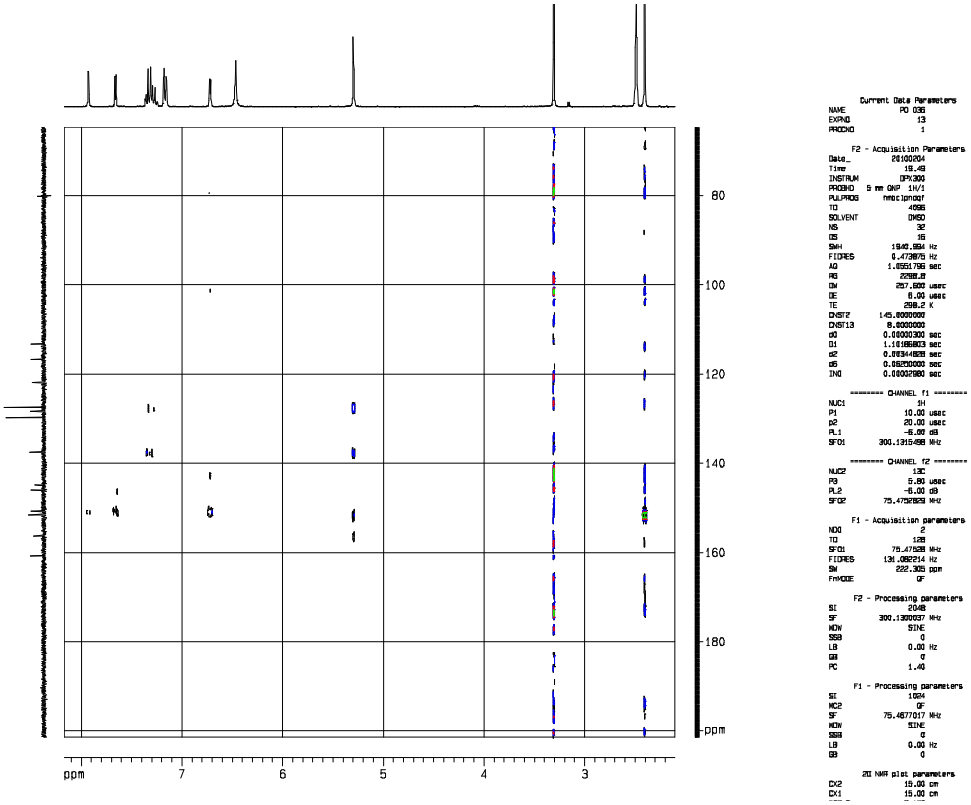
Spectrum 50. ^{1}H NMR of 9-benzyl-6-(furan-2-yl)-8-methyl-9*H*-purin-2-amine **(44a).**



Spectrum 51. ^{13}C NMR of 9-benzyl-6-(furan-2-yl)-8-methyl-9*H*-purin-2-amine **(44a).**

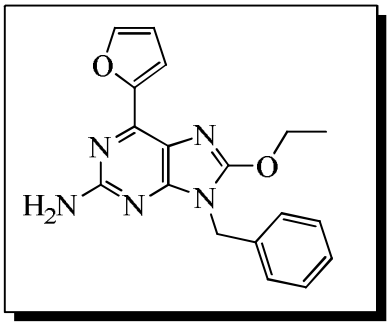


Spectrum 52. HMQC of 9-benzyl-6-(furan-2-yl)-8-methyl-9*H*-purin-2-amine **(44a).**



Spectrum 53. HMBC of 9-benzyl-6-(furan-2-yl)-8-methyl-9*H*-purin-2-amine **(44a).**

4.17. 9-benzyl-8-ethoxy-6-(furan-2-yl)-9*H*-purin-2-amine (45)



45

A mixture of 9-benzyl-8-chloro-6-(furan-2-yl)-9H-purin-2-amine (**42a)** (87 mg, 0.27 mmol) in a 0.2 M solution of sodium ethoxide in ethanol 10 mL was stirred at reflux under N2-atom for 15 hr, cooled, and poured into cold saturated aq NH_4CL (10 mL). Most of the ethanol was removed by evaporation in vacuo and the aqeous mixture was extracted with EtOAc (4 X 20

mL). The combined organic layers were dried ($MgSO_4$) and evaporated *in vacuo*. The product was purified by flash chromatography on silica gel eluting with EtOAc/hexame (1:1)

Yield: 57 mg, (63 %), Yellowish powder. M.p 167 – 170 ^oC

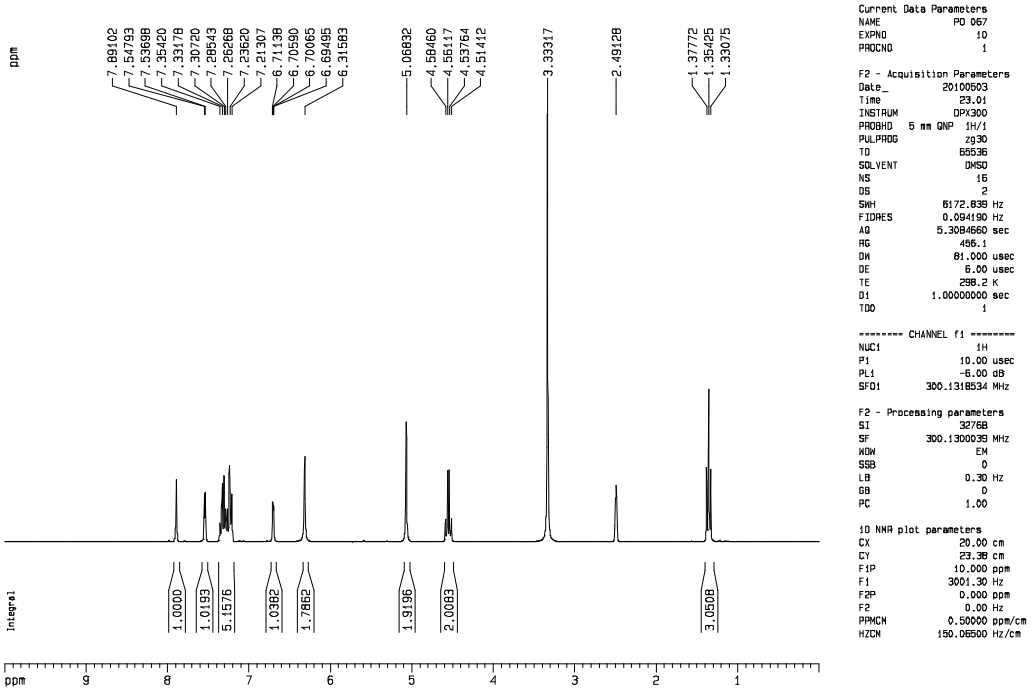
^{1}H NMR (DMSO- d_6, 300 MHz): δ 1.14 (t, J = 7.1 Hz, 3H, CH_3), 4.55 (q, J = 7.1 Hz, 2H, CH_2), 5.07 (s, 2H, CH_2), 6.32 (s, 2H, NH_2), 6.70 (dd, *J* = 3.3, 1.7Hz, 1H, H-4 in furyl), 7.21 – 7.35 (m, 5H, Ph), 7.54 (d, *J* = 3.3, 2.7Hz, 1H, H-3 in furyl), 7.89 (s, 1H, H-5 in furyl).

^{13}C NMR (DMSO- d_6, 75 MHz): δ 15.17 (CH_3), 44.39 (CH_2), 66.93 (CH_2), 113.06 (C-4 in furyl), 115.80 (C-3 in furyl), 119.48 (C-5), 127.87, 128.43, 129.53 (CH in Ph), 137.37 (C in Ph), 142.41 (C-6), 145.56 (C-5 in furyl), 150.65 (C-2 in furyl), 154.87 (C-4), 156.27 (C-8), 159.91 (C-2).

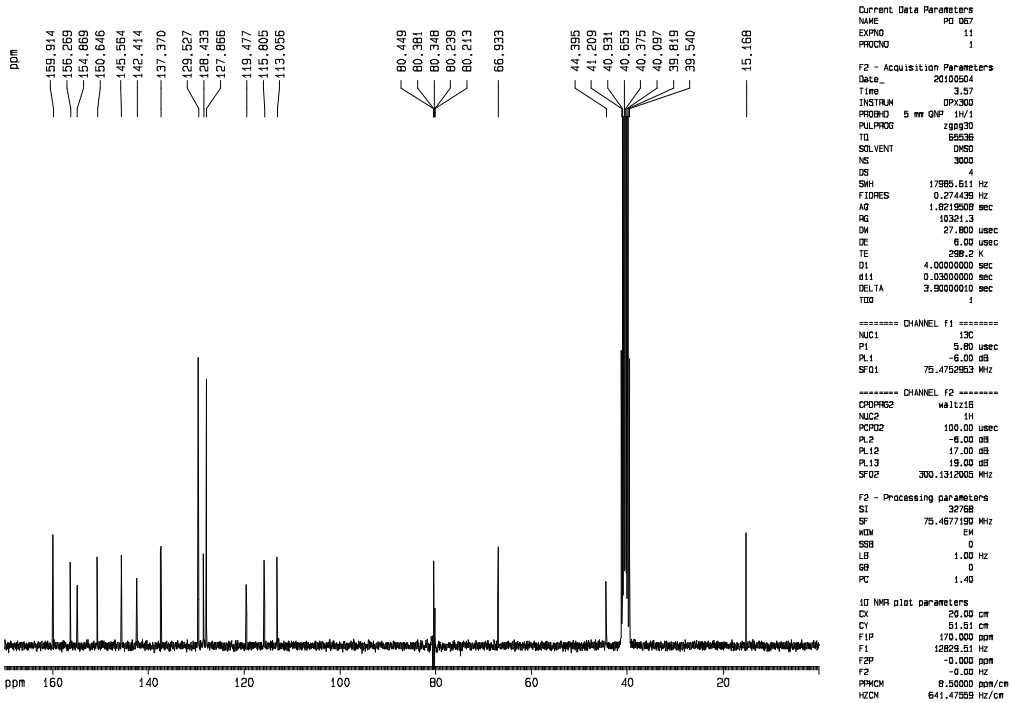
MS (EI) m/z (rel. %)**:** 335 (100, M^+), 91(92)

HR-MS: Found 335.137728, caculated value for $C_{18}H_{17}N_5O_2$ 335.1382

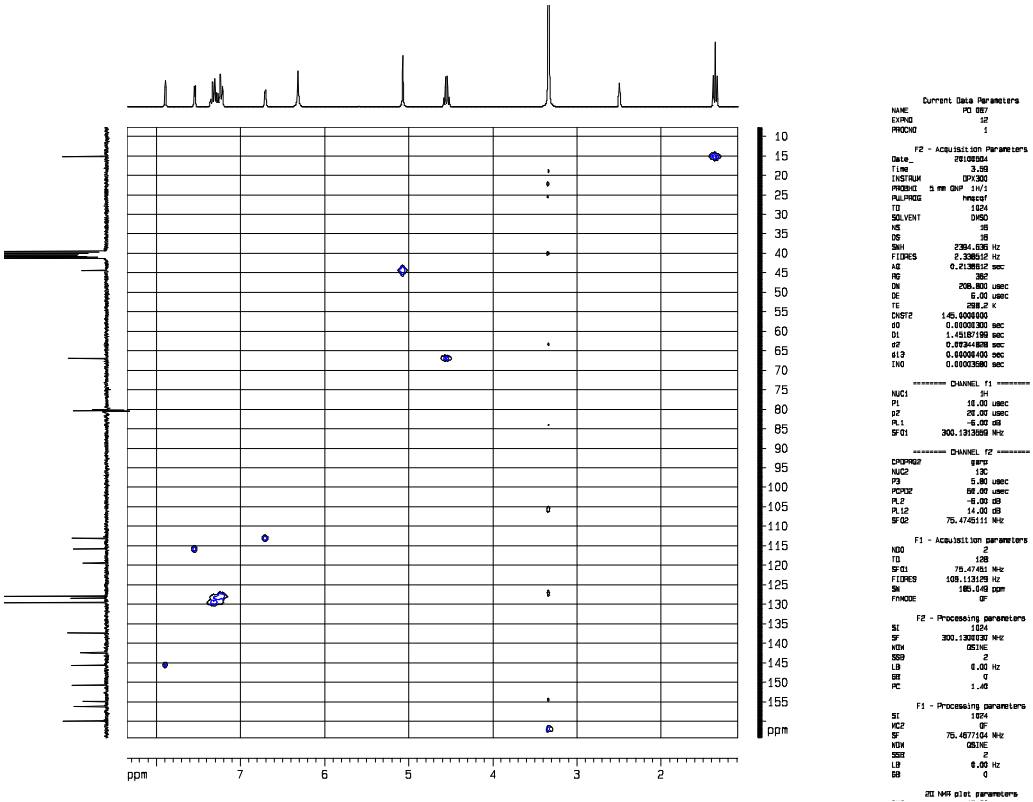
Anal: Found C, 64.38; H, 4.68; N, 21.07. $C_{18}H_{17}N_5O_2$ requires C, 64.47; H, 5.11; N, 20.88.



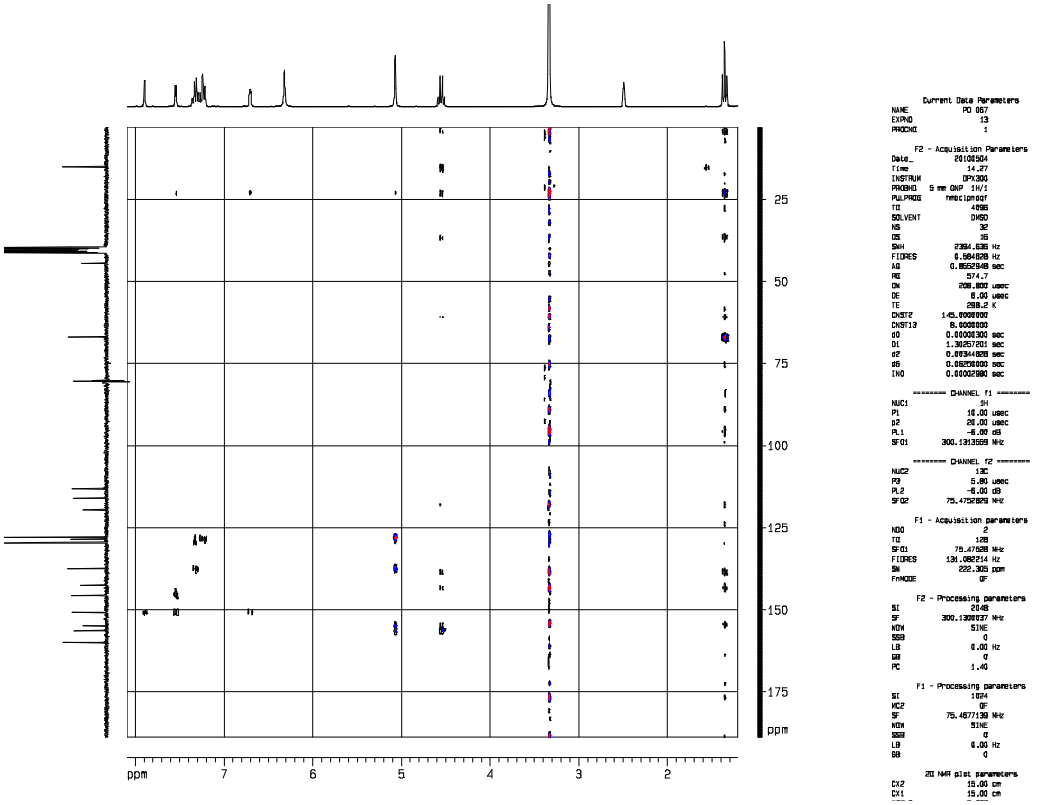
Spectrum 54. ^{1}H NMR of 9-benzyl-8-ethoxy-6-(furan-2-yl)-9*H*-purin-2-amine **(45).**



Spectrum 55. ^{13}C NMR of 9-benzyl-8-ethoxy-6-(furan-2-yl)-9*H*-purin-2-amine **(45).**



Spectrum 56. HMQC of 9-benzyl-8-ethoxy-6-(furan-2-yl)-9*H*-purin-2-amine **(45).**



Spectrum 57. HMBC of 9-benzyl-8-ethoxy-6-(furan-2-yl)-9*H*-purin-2-amine **(45).**

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